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Thesis

USING MICRORNAS (MIRNAS) AS THERAPEUTIC AGENTS IN CANCER: TARGETING THE LINK BETWEEN THE DOWNREGULATION OF MIRNAS AND ONCOGENESIS

by

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B.S., University of California, San Diego, 2015

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requirements for the degree of

Master of Science

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USING MICRORNAS (MIRNAS) AS THERAPEUTIC AGENTS IN CANCER: TARGETING THE LINK BETWEEN THE DOWNREGULATION OF MIRNAS AND ONCOGENESIS

AJAY S. NATHAN

ABSTRACT

With the worldwide prevalence of cancer, this disease has constituted a major public health problem for many years without great success in the development of treatment. This is due in part to the large genetic heterogeneity that exists, even between cancers of the same class. The current standard of care focuses on targeting shared elements such as rapid cell division, yet treatments like chemotherapy have tremendous cytotoxic and off-target adverse effects. Recently, a different link has been discovered that is shared by cancers of diverse types: the disruption in the regulation and expression of microRNAs (miRNAs). miRNAs, produced endogenously from discrete genes that exist for them, have been shown to be master regulators of diverse processes, including growth and division. They serve to downregulate or silence the expression of certain genes at the post-transcriptional level, with each miRNA regulating multiple target messenger RNAs. This provides an important therapeutic potential because the correction in the expression of any one miRNA, especially if it has tumor-suppressive functions, has the ability to inhibit cancer formation, growth, and metastasis by multiple related pathways.

The mechanisms by which the expression of miRNA is altered in cancer are reciprocal; that is, the processes that lead to oncogenesis alter the production of miRNA

and altered production of miRNA can lead to increased tumorigenicity. In particular, it has been found that miRNA is globally downregulated in most cancers. The downregulation of miRNA can arise incidentally because of chromosomal abnormalities that occur with cancer, or miRNA can be directly repressed through transcriptional control, epigenetic modification, or disruption in the biogenic production process. When miRNAs are downregulated, the condition can contribute to the main hallmarks of cancer: uncontrolled growth, angiogenesis, metastasis, and evasion of anti-growth and apoptotic signals.

A therapeutic strategy that has been investigated in numerous preclinical studies is miRNA replacement therapy, and this treatment has been shown to be extremely efficacious with a wide range of candidates and targets in diverse cancer systems. However, these advances have only led to two clinical trials, partly because of the lack of efficacious delivery methods. Nevertheless, with the rapid advances being made in gene therapy to address these shortcomings, a translation of these benefits from the bench-to-bedside is almost certain.

TABLE OF CONTENTS

TITLE……...i

COPYRIGHT PAGE……...ii

READER APPROVAL PAGE……..iii

ABSTRACT..... iv

TABLE OF CONTENTS..... vi

LIST OF TABLES..... viii

LIST OF FIGURES ix

LIST OF ABBREVIATIONS..... x

INTRODUCTION 1

Importance of the Development of Molecular Targeted Cancer Therapies..... 1

Biogenesis Endogenous miRNA..... 3

The Mechanism of Action of miRNA 6

Objectives 10

MECHANISMS OF miRNA DOWNREGULATION IN CANCER 11

Incidental Reduction Due to Chromosomal Position..... 11

Dysregulation of Transcriptional Control..... 12

Dysregulation in Epigenetic Modifications 14

Dysfunction in miRNA Biogenesis 17

MECHANISMS OF ONCOGENESIS FROM DOWNREGULATED miRNA..... 20

Uncontrolled Growth: Self-Sufficiency in Growth Signals..... 20

Evasion of Apoptotic Signals..... 23

Tissue Invasion and Metastasis..... 27

Inducing Sustained Angiogenesis..... 29

REPLETION OF DOWNREGULATED miRNAs IN CANCER MODELS 31

Potential Target miRNAs Downregulated by Cancer..... 31

Overview of Preclinical Models for miRNA Replacement Therapy..... 33

DEVELOPMENT OF miRNA THERAPEUTICS IN CANCER 41

Challenges and Strategies in the Use of miRNAs as Therapeutic Agents..... 41

Clinical Trials in miRNA Replacement in Cancer 45

FUTURE DIRECTIONS AND CONCLUSIONS..... 48

The Potential for miRNA in Gene Silencing 48

Conclusions..... 49

APPENDIX..... 51

REFERENCES 64

CURRICULUM VITAE..... 76

LIST OF TABLES

Table	Title	Page
1	Downregulated miRNAs Linked to Cancers by System	32
2	Survey of Preclinical Studies in miRNA Replacement Therapy	34

LIST OF FIGURES

Figure	Title	Page
1	Nuclear and cytoplasmic events in miRNA biogenesis.	5
2	miRNA biogenesis and links to cancer.	19
3	Overview of the downstream effects of IGF-IR.	22
4	Intrinsic and extrinsic pathways of apoptosis.	25
5	Strategies in miRNA replacement therapy.	42
6	Molecular targets of miRNAs in clinical development.	46

LIST OF ABBREVIATIONS

Ago.....Argonaute

Akt..... protein kinase B

AML1..... acute myelogenous leukemia 1

Apaf-1 .....apoptotic protease-activating factor 1

BAD .....Bcl-2-associated death promoter

BamH 1 ..... Bacillus amyloliquefaciens H 1

Bcl.....B-cell lymphoma

BMI1 ..... B-cell-specific Mo-MLV insertion site-1

CCR4-NOT..... carbon catabolite-repression 4-Not

CDC25A .....cell division cycle 25A

CDK .....cyclin-dependent kinase

CLL .....chronic lymphocytic leukemia

CMV .....cytomegalovirus

CNS.....central nervous system

CpG .....5'—cytosine—phosphate—guanine—3'

DGCR8 .....DiGeorge syndrome critical region 8

DISC ..... death-inducing signaling complex

DNMT..... DNA methyltransferase

DR.....death receptor

ds.....double-stranded

dsRNA..... double-stranded RNA

EDV .....EnGeneIC Dream Vector

EGFR .....epidermal growth factor receptor

eIF .....eukaryotic initiation factor

EMT .....epithelial-to-mesenchymal transition

ERK.....extracellular signal-regulated kinase

ETO.....eight twenty-one

FADD..... Fas-associated protein with death domain

Fas..... first apoptosis signal

FLIP .....FLICE-like inhibitory protein

FOXO..... forkhead box O

GSK3.....glycogen synthase kinase 3

GTP.....guanosine triphosphate

HDAC .....histone deacetylase

HIF ..... hypoxia-inducible factor

IAP ..... inhibitor of apoptosis

IGF .....insulin-like growth factor

IGF-IR.....insulin-like growth factor-I receptor

LDHA ..... lactate dehydrogenase A

let.....lethal

MAPK..... mitogen-activated protein kinase

MDM2..... murine double minute 2

MET ..... mesenchymal-to-epithelial transition

miR ..... microRNA

miRISC ..... miRNA-induced silencing complex

miRNA ..... microRNA

MLKL ..... mixed lineage kinase domain-like

mRNA ..... messenger RNA

mTOR ..... mammalian target of rapamycin

nt ..... nucleotide

PABP ..... poly(A)-binding protein

P-body ..... processing body

PDL ..... programmed death ligand

PGAM5 ..... phosphoglycerate mutase family member 5

PI3K ..... phosphoinositide 3-kinase

PKR ..... protein kinase R

pol ..... polymerase

poly(A) ..... polyadenylated

pre-miRNA ..... precursor miRNA

pri-miRNA ..... primary miRNA

RIP ..... receptor-interacting protein

RISC ..... RNA-induced silencing complex

RNase ..... ribonuclease

SNAI ..... snail

SOS .....son of sevenless

Sv40 .....simian virus 40

TERT..... telomerase reverse transcriptase

TGF-β.....transforming growth factor-β

TNF .....tumor necrosis factor

TNFR ..... tumor necrosis factor receptor

TRADD..... TNFR type 1-associated death domain

TRAF2 ..... TNFR-associated factor 2

TRAIL..... TNF-related apoptosis-inducing ligand

TRBP..... transactivation-responsive RNA-binding protein

VEGF .....vascular endothelial growth factor

ZEB .....zinc-finger E-box-binding homeobox

INTRODUCTION

This thesis provides a review of microRNA (miRNA) and how its downregulation is involved in oncogenic pathways in order to judge the potential for using miRNA replacement as a treatment of cancer. Candidates with tumor-suppressive function validated through preclinical studies are discussed, as well as those that have progressed into clinical trials. Finally, future directions for artificially designed miRNA therapeutics in cancer are considered. The following introduction provides background information on the importance of research on new targeted drugs in this field, the biogenesis of miRNAs, and the mechanism of action of miRNAs.

Importance of the Development of Molecular Targeted Cancer Therapies

As one of the most common diseases, cancer places an extremely large burden on societies worldwide. There were 14.1 million new cases of cancer globally and 8.2 million mortalities in 2012. These statistics are only expected to rise in the coming years because of the aging society and the growing environmental and lifestyle contributions to cancer (Torre et al., 2015). Thus, there is a continuing need for research and development of new treatments in this field.

The current treatment of cancer is focused on a multidisciplinary treatment plan approached through a combination of major treatment methods such as surgery, radiation therapy, and chemotherapy (Chan, 2007). However, cytotoxic chemotherapeutic agents, which kill rapidly dividing cells, have many drawbacks (Sun et al., 2017). Their low

selectivity causes many adverse off-target effects and long-term health issues, and continued use has been associated with drug resistance and loss of efficacy.

Oncogenesis is a multistep process involving the accumulation of multiple genetic abnormalities. Recent advances in the understanding of molecular mechanisms of these processes, such as the pathways that regulate cell growth, have ushered in a new era of targeted therapies (Schultz, 2005). Although no one therapy will be able to treat all types of cancer, the discovery of particular molecular mechanisms and signatures of cancer will aid in the development of novel therapies to specifically treat a patient's cancer. The approval of targeted cancer therapies has soared in the last decade from being far lower than cytotoxic agents to being five times higher. Most of these agents are targeted against cell membranes or enzymes such as tyrosine kinase (Sun et al., 2017). With the rapid growth of the field of targeted cancer chemotherapies, the subcategory of gene therapy (treatment involving introduction of exogenous DNA or RNA) is emerging as well.

The discovery of microRNAs (miRNAs) approximately 20 years ago and the ensuing research into their role in post-transcriptional gene regulation have caused a new excitement in the field of gene therapy. Subsequently, miRNAs have been linked to many cancers and thus could hold great promise as a therapeutic strategy in this disease. miRNAs are non-coding RNAs of about 22 nucleotides (nts) in length (Fabian, Sundermeier, & Sonenberg, 2010) that serve to regulate gene expression for a variety of pathways, including cell proliferation and cell death (Brennecke, Hipfner, Stark, Russell, & Cohen, 2003). Because miRNAs serve to regulate many processes, downregulation of their pathways can critically accelerate oncogenic transformation (Kumar, Lu, Mercer,

Golub, & Jacks, 2007). It has been shown that miRNA normally functions to prevent cell division and drive terminal differentiation. Thus downregulation of any one miRNA pathway can lead to stem cell-like growth, that is, continuing division without differentiation (Lu et al., 2005). In these cases, miRNA synthetically developed to mimic the endogenous miRNA (i.e., miRNA mimics) can be used to replenish the product of the downregulated pathway. This treatment, being investigated in numerous preclinical studies and a few clinical trials to date, shows potential as a curative treatment of these cancers.

This thesis primarily evaluates and reviews developments in miRNA replacement therapy in which miRNA mimics are used to treat miRNA downregulation in cancers. Artificially designed miRNA also holds promise in another strategy for cancer therapeutics. Targeting specific genes can be difficult because many different types of genomic abnormalities accumulate in cancer cells and large genomic heterogeneity can exist even between cancers of the same class (The Cancer Genome Atlas Research Network, 2008). Since a single miRNA has the capability to silence multiple related genes, it has the potential to suppress the multiple oncogenic pathways that lead to cancer (Bader, Brown, Stoudemire, & Lammers, 2011).

Biogenesis Endogenous miRNA

Nuclear Processing

Separate genes forming independent transcriptional units encode for miRNAs, although some miRNAs can derive from introns processed from genes that code for

proteins (Fabian et al., 2010). These genes are transcribed by RNA polymerase II (Y. Lee et al., 2004) or RNA polymerase III (Borchert, Lanier, & Davidson, 2006) to produce long hairpin-loop primary miRNA (pri-miRNA) transcripts that are processed in the nucleus by a multifunctional enzyme complex termed the microprocessor. The microprocessor consists of two components: the ribonuclease III (RNase III) endonuclease Drosha and the protein DiGeorge syndrome critical region 8 (DGCR8) that binds double-stranded (ds) pri-miRNA (R. I. Gregory et al., 2004). The pri-miRNA is cleaved at the 5′ and 3′ ends by the microprocessor to form the 60-70-nt precursor miRNA (pre-miRNA), which then folds into a stem-loop hairpin structure. This is exported into the cytoplasm by means of exportin-5 (Lund, Güttinger, Calado, Dahlberg, & Kutay, 2004) in the presence of the Ran-guanosine triphosphate (GTP) cofactor (Yi, Qin, Macara, & Cullen, 2003).

Cytoplasmic Processing

Once in the cytoplasm, pre-miRNA is further processed by Dicer, another RNase III enzyme that functions as a part of the RNA-induced silencing complex (RISC)-loading complex. The RISC-loading complex also contains a double-stranded RNA (dsRNA)-binding protein called transactivation-responsive RNA-binding protein (TRBP), a protein activator of protein kinase R (PKR), and Argonaute (Ago) proteins. Not only are Ago proteins involved in the loading process, but Ago2 is particularly important in strand selection and for later post-transcriptional regulation. Dicer cleaves the loop portion of the pre-miRNA and forms 22-nt ds miRNA. The ds miRNA is unwound by various helicases, and the accessory proteins dissociate from the RISC-

loading complex. This leads to the formation of the miRNA-induced silencing complex (miRISC), which contains a single strand of guide miRNA (Fabian et al., 2010). The complete process is illustrated in **Figure 1**.

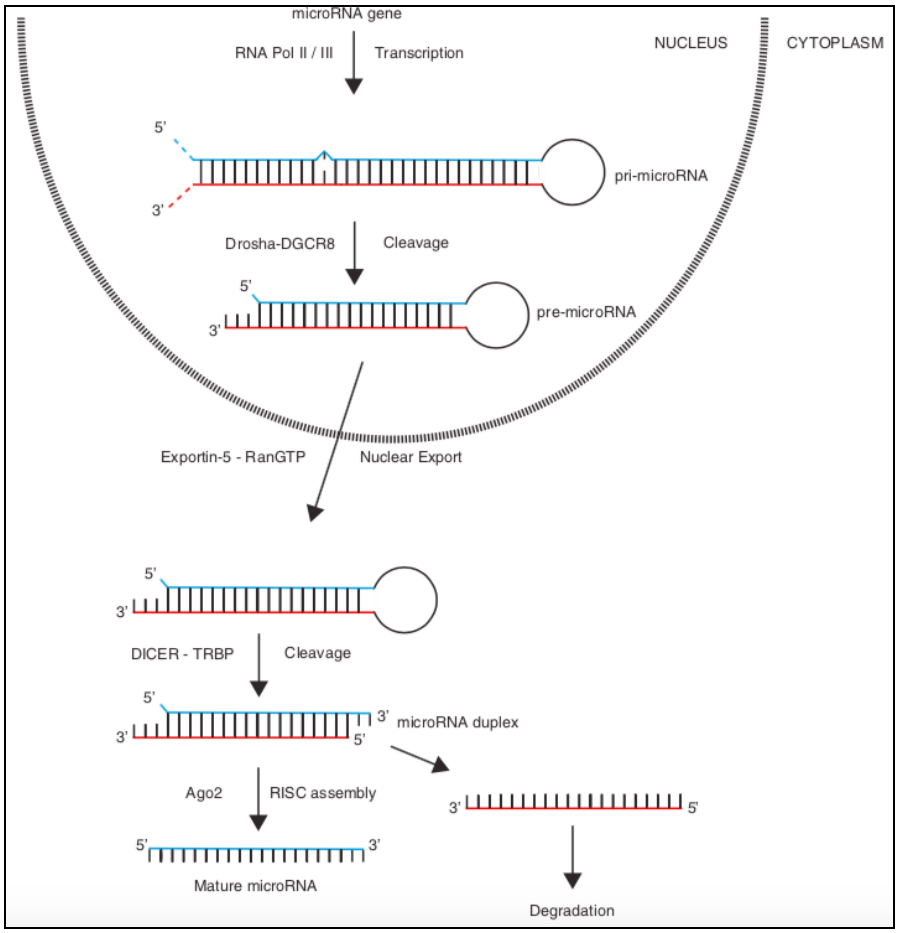

Figure 1. Nuclear and cytoplasmic events in miRNA biogenesis. miRNAs are transcribed from genes by RNA polymerase II or III to form pri-miRNA in the nucleus, which is processed by Drosha, a type III RNase, to form pre-miRNA. Exportin-5/Ran-GTP transports pre-miRNA by means of nuclear pores to the cytoplasm, where it is further processed by Dicer. Single-stranded mature miRNA is loaded onto RISC and used as a guide for translation repression and mRNA degradation. Ago2 = Argonaute 2; DGCR8 = DiGeorge syndrome critical region 8; GTP = guanosine triphosphate; miRNA = microRNA; mRNA = messenger RNA; pol = polymerase; pre-miRNA = precursor microRNA; pri-miRNA = primary microRNA; RISC = RNA-induced silencing complex; RNase = ribonuclease; TRBP = transactivation-responsive RNA-binding protein. Figure taken from (Fabian et al., 2010).

The Mechanism of Action of miRNA

There are two ways in which miRNA acts to limit gene expression: by degrading or destabilizing messenger RNA (mRNA) or by directly disrupting the process of translation. There are no sequences of miRNA that are fully complementary to a human gene, and thus the pathway of post-transcriptional gene repression is determined through imperfect binding with and the degree of complementarity in key sites between the guide miRNA and the mRNA (Hutvágner & Zamore, 2002). These two methods of miRNA control are not mutually exclusive but rather can act simultaneously through distinct mechanisms of action (Fabian et al., 2010). The extent to which each mechanism plays a role is dependent on the system and is highly controversial. Nevertheless, there is some evidence to show that translation repression is the more central mechanism and occurs temporally before mRNA degradation (Bazzini, Lee, & Giraldez, 2012).

Translational Repression

Translation is a multistep process requiring many cofactors, and it can be divided into three main stages of initiation, elongation, and termination. miRNA can block translation at either the initiation stage or some stage after initiation (Fabian et al., 2010). However, in general, the mechanisms presented focus on regulation at the initiation stage because it is the most upstream process and appears to be the most common method of repression. Thus, the following paragraph provides a brief description of this stage with the details pertinent to this discussion.

Translation Initiation. Prior to translation initiation, protein-coding genes are transcribed in the nucleus into mRNA. The mRNA is modified post-transcriptionally to add a 7-methylguanosine triphosphate cap (in addition to 3′ polyadenylation and intron splicing) that protects it and allows for translation initiation. Translation occurs in the cytoplasm, and cap-dependent initiation begins with the recruitment of required eukaryotic initiation factors (eIFs). These eIFs form a multifunctional complex that associates with the cap, and the complex then recruits the small ribosome subunit to associate with mRNA near the 5′ cap. This complex travels in the 5′ to 3′ direction scanning for the start codon contained in the Kozak sequence. The 3′ polyadenylated [poly(A)] tail associates with a poly(A)-binding protein (PABP) and the eIF complex to enhance translation initiation, forming a loop-like structure in the mRNA. After the Kozak sequence is reached, GTP is hydrolyzed with another eIF, the large subunit associates, and elongation of the polypeptide can occur (Fabian et al., 2010).

There are many studies to show that translation inhibition occurs at the initiation step. For example, if eIF factors are mutated, the translation inhibition by way of miRNA is antagonized (Mathonnet et al., 2007). This leads to the conclusion that early steps, such as eIF interaction with the mRNA cap, are points of translation initiation repressed by miRNA. It has also been suggested that miRNAs can impede the formation of the loop-like structure in mRNA, which would cause a weaker association between eIFs and the cap (Fabian et al., 2010). The mechanisms by which miRNAs inhibit translation are not fully understood, especially in humans. However, these examples provide some idea of

how miRNAs can disrupt the process of translation and lead to post-transcriptional gene regulation.

mRNA Destabilization

Although miRNA disrupts the process of translation, mRNA destabilization also plays a major role in the downregulation of genes. In one study, it was found that even though translation was inhibited, there was a 50% decrease in the amount of target RNA (Petersen, Bordeleau, Pelletier, & Sharp, 2006). There are many proposed mechanisms for the observed mRNA repression. One study has suggested that miRNA functions through deadenylating the poly(A) tail (Giraldez et al., 2006). The poly(A) tail provides stability to the mRNA, and when the poly(A) tail is removed, mRNA decay is enhanced. The poly(A) tail, as discussed in the previous section, is also involved with translation initiation and thus could function in this way to repress translation initiation. However, deadenylation directly mediated by miRNA is more responsible for target mRNA degradation than translation repression alone (Giraldez et al., 2006). Indeed, it has been shown that there is significant miRNA-mediated mRNA degradation even in the presence of translation inhibitors (Eulalio et al., 2007), which strongly suggests that an independent mechanism for mRNA degradation exists.

Central to the process of direct degradation of mRNA is Ago2. miRNA is known to contain "seed" regions that are used for target recognition and complementarity (Lai, Tam, & Rubin, 2005). When an mRNA is sufficiently complementary to the miRNA seed sequence, it can be directly cleaved by the endonuclease activity of Ago2 (Liu et al., 2004). However, in humans, this level of complementarity is rare, and thus another

mechanism of mRNA degradation could play a more central role. There are portions in the cytoplasm known as processing bodies (P-bodies) which contain mRNA degradation enzymes. There is evidence to show that Ago proteins colocalize with mRNA targets to these P-bodies to stimulate mRNA degradation (Meister et al., 2005). P-bodies also contain the carbon catabolite-repression 4-Not (CCR4-NOT) complex which deadenylates mRNAs, decapping factors, and other translational repressors that could all serve to destabilize or impede the functioning of mRNAs (Behm-Ansmant et al., 2006).

Objectives

This thesis aims to:

1. Review the reciprocal mechanisms by which downregulated miRNA is linked to oncogenesis.
2. Provide an overview of downregulated miRNAs in cancers and a general survey of the current miRNA replacement therapy field in preclinical studies.
3. Assess the challenges and strategies involved with using miRNA mimics as therapeutic agents, discuss the potential targets, and evaluate the efficacy of those mimics that have progressed to clinical trials.
4. Discuss the potential use of miRNA therapeutic agents for gene silencing and conclude with future directions of miRNAs in cancer therapeutics.

MECHANISMS OF miRNA DOWNREGULATION IN CANCER

Certain miRNAs are upregulated in some cancers, and in these cases, they are termed onco-miRNAs in that they promote oncogenesis. However, cancer is generally associated with loss of function of miRNA regulation. When diverse cancers were profiled, miRNA expression was found to be globally downregulated (Lu et al., 2005). There are many mechanisms by which miRNA can be downregulated in cancer. The following sections are a review of the ways in which miRNAs can become downregulated in the process of oncogenesis.

Incidental Reduction Due to Chromosomal Position

The genes for miRNAs are located in fragile areas of DNA, or breakpoints, and these positions make them susceptible to loss through DNA damage (Calin et al., 2004). As a consequence of the deleted regions and deleted miRNA genes, reduced miRNA expression has been reported. In a genome-wide association study of breast cancer, ovarian cancer, and melanoma, it was found that there were a high number of miRNA copy number alterations in these cancers and that the alteration of miRNA genes was an important part of miRNA dysregulation and oncogenesis (Zhang et al., 2006). Even at the genomic level of a diverse range of cancers, the strong association between miRNA copy number alterations and the development of cancer has been linked.

Although certain miRNA downregulations are common to many different cancers, each type is expected to have a signature in the copy number and expression levels of a

specific miRNA (Zhang et al., 2006). For example, evidence can be used from one genotypic study performed on 60 patient samples with chronic lymphocytic leukemia (CLL). CLL, the most common adult leukemia, results from hemizygous or homozygous chromosomal loss at 13q14 in more than half of the subtypes and is often the only genetic aberration. It can be inferred that genes in this location contain some tumor suppressor function. In this region, miRNA-15 and miRNA-16, two miRNA genes, were found to be deleted as well, and on evaluation of the expression levels, they were found to be low in 68% of the CLL patients (Calin et al., 2002). Thus, it is clear that there is a link between the genetic abnormalities that cause oncogenesis and the expression of miRNA.

Dysregulation of Transcriptional Control

The expression of miRNA is regulated through transcription factors, and certain malignancies can directly interfere with the normal function of transcription factors, reducing transcription of miRNA genes. This mechanism is increasingly being linked to the development of disease (Mraz, Pospisilova, Malinova, Slapak, & Mayer, 2009). One mechanism that has been proposed is direct binding to the promoter elements of the miRNA genes. A well-documented example is c-myc, which is classified as a proto-oncogene. A proto-oncogene is a normal gene that, when mutated, contributes to the development of cancer. Its gene product, c-Myc, is a transcription factor that regulates the cell cycle, apoptosis, growth, and metabolism. When c-Myc is overexpressed, it can drive proliferation; thus, it is considered a hallmark of many cancers (Dang et al., 1999).

The miRNA-17 cluster has been identified as the target of c-Myc. In malignancies in which c-Myc is activated, 6 miRNAs of this cluster are activated as well. The downstream effect of c-Myc is E2F1 activation, which drives progression of the cell cycle (O'Donnell, Wentzel, Zeller, Dang, & Mendell, 2005). Although c-Myc activates oncogenic miRNAs, only two of them show statistically significant overexpression. It was found that c-Myc more significantly represses a diverse group of tumor-suppressive miRNAs. These include miRNA-15a, miRNA-22, miRNA-26, miRNA-29, miRNA-30, and lethal (let)-7 families. There is evidence to show that c-Myc directly binds to sites upstream of the gene on the 5′ end of the pri-miRNA. This provides verification that c-Myc binds to promoters for these miRNA genes and inhibits them, thus repressing their transcription (Chang, Yu, et al., 2007). An example of this mechanism of promoter repression occurs in hepatocellular cancer, in which c-Myc directly represses miRNA-122. However, in this case, c-Myc also functions to suppress the endogenous transcription factor of miRNA-122. Finally, c-Myc is reciprocally repressed through miRNA-122. The disruption of this pathway is the critical aspect of the oncogenic pathway of hepatocellular carcinoma (Wang et al., 2014).

Another important transcription factor that is involved with cancer is p53, a protein which controls the cell cycle, differentiation, DNA repair, and apoptosis (Olivier, Petitjean, Fromentel, & Hainaut, 2010). p53 is encoded by the tumor suppressor gene TP53, which is considered to be a gene of essential importance that, when mutated, leads to oncogenesis. Loss of function of the p53 transcription factor leads to release from replication inhibition under times of cellular stress, and with reduced ability for DNA

repair, cells accumulate mutations that result in cancer. In fact, mutations in TP53 are the most common alterations across all cancer types (Olivier et al., 2010). One example of its association with miRNA regulation is the p53-miRNA-34 regulatory axis. The miRNA-34 gene is found to be deleted in many cancers. The p53 transcription factor directly induces the transcription of the miRNA-34 family by means of the previously described mechanisms to yield some of the tumor-suppressive effects, such as cell-cycle arrest and apoptosis (Chang, Wentzel, et al., 2007). In other studies, p53 has been similarly shown to induce the production of miRNA-605 (Xiao, Lin, Luo, Luo, & Wang, 2011), miRNA-1246 (Zhang, Liao, Zeng, & Lu, 2011), and miRNA-107 (Yamakuchi et al., 2010).

In summary, oncogenic proteins can directly repress miRNA production, which drives the cell toward cancer, especially when the repressed miRNAs have tumor-suppressive functions.

Dysregulation in Epigenetic Modifications

Epigenetic modifications are a hallmark of cancer and can be used to distinguish between normal and cancerous cells. A large amount of hypomethylation is one such epigenetic feature that is found in cancer (Feinberg & Vogelstein, 1983). The presence of hypomethylation on oncogenes increases their level of transcription. Tumor suppressor genes can also be silenced by hypermethylation on the 5′ regulatory region of genes (Herman et al., 1994). Methylation as a regulatory mechanism appears to only play a role in a minor portion of overall miRNA regulation (about 10% in a model of colon cancer). However, for those miRNAs controlled through DNA methylation, this method of

regulation has a major effect on the expression of miRNA (Han, Witmer, Casey, Valle, & Sukumar, 2007).

An example of this process is illustrated in acute myelogenous leukemia. Resulting from a chromosomal translocation, the most common fusion protein, acute myelogenous leukemia 1 (AML1)/eight twenty-one (ETO), is formed. This protein directly targets miRNA-223; however, rather than inhibiting its transcription, it recruits chromatin remodeling enzymes to induce heterochromatic silencing (Fazi et al., 2007). The mechanism for silencing is as follows: (1) AML1/ETO fusion protein recruits a combination of histone deacetylases (HDACs), which allow histones to associate more tightly with DNA and repress transcription, and DNA methyltransferases (DNMTs) (S. Liu et al., 2005). (2) DNMTs methylate the cytosines on "CpG islands," sites of transcription regulation located upstream of the gene, in which a cytosine is followed by a guanine in the linear sequence of bases. (3) DNA methyl-CpG-binding proteins associate with methylated CpGs, inhibit transcription through repressors, and modulate chromatin remodeling (Ng et al., 1999). Expression levels of miRNA-223 can be restored with a demethylating treatment that allows the cell to return to normal cycles of replication and differentiation (Fazi et al., 2007).

Expression of 17 out of 313 human miRNAs is greatly upregulated in the presence of demethylating agents or HDAC inhibitors in transitional carcinoma cells compared with normal cells (Saito et al., 2006). In particular, miRNA-127 is located within a CpG island and is expected to be highly regulated by epigenetic modifications. The expression of miRNA-127 is silenced in human cancer cells. Indeed, miRNA-127

was found to be downregulated in tumors of the bladder, prostate, and colon, whereas it was highly expressed in the corresponding normal tissue (Saito et al., 2006). One target of miRNA-127 is the proto-oncogene B-cell lymphoma 6 (BCL6), and thus downregulation of the miRNA releases the inhibition on expression of BCL6, which contributes to oncogenesis. Upon introduction of exogenous miRNA-127, it was found that BCL6 was inhibited, indicating that a potential treatment of this nature could be efficacious in therapy (Saito et al., 2006). Similarly, the effect of a DNA-demethylating agent on miRNA expression was tested in lymph node metastatic cancer cells (Lujambio et al., 2008). The miRNAs found to be upregulated following this treatment were miRNA-148a, miRNA-34b/c, and miRNA-9, which are known to undergo hypermethylation-related silencing in cancer. Most important, this study found that restoration of expression of these miRNAs could be efficacious in inhibiting metastasis (Lujambio et al., 2008).

Other studies have elucidated cancers in which epigenetic inactivation leads to downregulated miRNA. In breast cancer, there is epigenetic silencing of miRNA-9 (Lehmann et al., 2007), and in lung (Donzelli et al., 2015) and colon cancers (Wang et al., 2016), miRNA 145-5p is silenced. These studies illustrate that epigenetic transcription repression is a major mechanism in miRNA downregulation in cancer and thus could be an important factor in the development of future therapies.

Dysfunction in miRNA Biogenesis

As previously described, miRNA biogenesis is a complex process involving a number of steps and enzymes. Cancer can involve the mutation or the repression of function of any of the involved enzymes or transporters, leading to reduced production of miRNA. In some cases, miRNA downregulation in cancer can occur during the miRNA processing inhibition at the Drosha step, where pri-miRNA is cleaved (Thomson et al., 2006). In an analysis of Wilms tumors, 15% displayed mutations in DGCR8 and Drosha that resulted in decreased expression of let-7a and the microRNA (miR)-200 family of miRNAs (Walz et al., 2015). Dicer was found to be impaired in colorectal cancer, and the resulting low miRNA levels contributed to an enhanced tumor stem-like nature and metastatic potential (Iliou et al., 2013). These dysfunctions in miRNA biogenesis enzymes have direct correlation with patient outcomes. In an analysis of 111 specimens from patients with invasive epithelial ovarian cancer, the levels of Dicer and Drosha were found to be decreased in over half the samples. Low Dicer levels significantly correlated with advanced tumor stages. Higher levels of both enzymes significantly correlated with increased survival (Merritt et al., 2008). Similar correlations were found in a study conducted on patients with lung cancer (Karube et al., 2005).

Cancer can disrupt miRNA biogenesis at the cytoplasmic stages involving RISC. In particular, the expression of Ago proteins, essential to the functioning of RISC, can be downregulated. In melanoma, Ago2 expression was found to be greatly reduced by means of post-transcriptional regulatory methods (Völler, Reinders, Meister, & Bosserhoff, 2013). Another step in the miRNA biogenesis machinery that can be affected

is exportin-5. This transporter mediates pre-miRNA export from the nucleus and can be inactivated because of genetic mutation in some types of cancer. A defect in exportin-5 would block pre-miRNAs in the nucleus, decreasing miRNA processing and therefore miRNA-mediated silencing (Melo et al., 2010).

All of the known changes in miRNA biogenesis leading to cancer are illustrated in **Figure 2.** These studies show that oncogenic processes can downregulate miRNA by impairment in the mechanisms of its biogenesis, and these processes have direct effects on the prognosis and severity of cancers (Merritt et al., 2008).

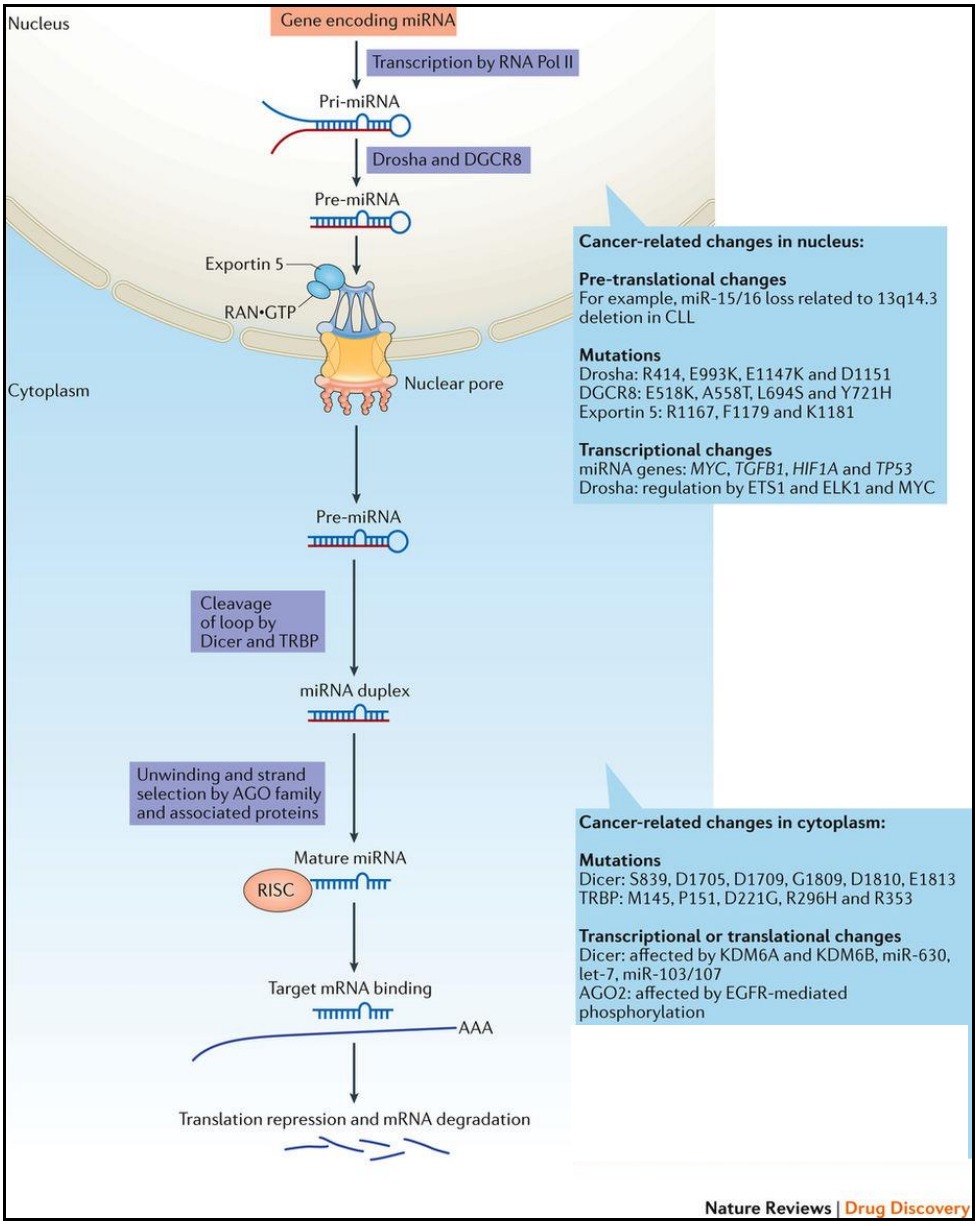

Figure 2. miRNA biogenesis and links to cancer. Defects in any step of the process in miRNA biogenesis can lead to decreased miRNA production. As shown in the figure, certain mutations and decreases in miRNA have been linked to cancer. Ago = Argonaute; CLL = chronic lymphocytic leukemia; DGCR8 = DiGeorge syndrome critical region 8; GTP = guanosine triphosphate; let = lethal; miR = microRNA; miRNA = microRNA; mRNA = messenger RNA; pol = polymerase; pre-miRNA = precursor miRNA; pri-miRNA = primary miRNA; RISC = RNA-induced silencing complex; TRBP = transactivation-responsive RNA-binding protein. Figure modified from (Rupaimoole & Slack, 2017).

MECHANISMS OF ONCOGENESIS FROM DOWNREGULATED miRNA

Hanahan and Weinberg (2000) set the currently accepted standard of six hallmarks that characterize the development of cancer. These features are: (1) self-sufficiency in growth signals, (2) evading apoptosis, (3) insensitivity to anti-growth signals, (4) tissue invasion and metastasis, (5) limitless replicative potential, and (6) sustained angiogenesis (Hanahan & Weinberg, 2000). The downregulation of miRNA can contribute to oncogenesis in each of these six ways. This section provides examples of how miRNAs can contribute to these hallmarks of cancer in order to understand how the reversal of downregulation or the repletion of these miRNAs can be used in treatment.

Uncontrolled Growth: Self-Sufficiency in Growth Signals

There are several signaling pathways involved with growth, proliferation, and differentiation that are regulated by miRNAs. Certain miRNAs involved with signaling pathways are upregulated in cancer, such as the previously described miRNA-17 cluster induction of E2F1 expression and the miRNA-221/222 targeting and downregulating of the expression of cyclin-dependent kinase (CDK) inhibitors (Gillies & Lorimer, 2007). Both of these miRNA upregulations drive the cell toward replication. However, one pathway that is involved with downregulated miRNA is the insulin-like growth factor (IGF) pathway. This pathway is engaged with normal growth and development, but its dysfunction is implicated with several disease states. The IGF pathway is mediated by

IGF-I and IGF-II, two proteins which activate separate receptors and downstream messengers. The downstream effects of IGF-I are especially important for its implication in oncogenesis. IGF-I binds to its receptor (IGF-IR, a receptor tyrosine kinase), which shares structural homology to the insulin receptor. Therefore, the signaling pathways for these messengers overlap to some extent. IGF-IR activates two signaling cascades: phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK). The PI3K and MAPK pathways are illustrated as an overview in **Figure 3**. The following description of these pathways has been adapted from the work of Ferguson and colleagues (2012).

In the PI3K pathway, there is recruitment of insulin receptor substrates that associate with PI3K and activate it. Once PI3K is activated, phospholipid phosphatidylinositol 3,4,5-trisphosphate in the plasma membrane increases, leading to the activation of protein kinase B (Akt) and its association with the membrane. Akt proceeds to activate several downstream effectors involved with metabolism, survival, migration, and proliferation. One important effector is the mammalian target of rapamycin (mTOR) complex 1, which induces growth, metabolism, and the production of protein by activation of an eIF. Akt also inactivates the cell-cycle arrest by promoting forkhead box O (FOXO) transcription factors and the apoptotic protein Bcl-2-associated death promoter (BAD). The inactivation of the glycogen synthase kinase 3 (GSK3) leads to anti-apoptotic and hypertrophic effects (Ferguson et al., 2012).

In the MAPK pathway, the guanine nucleotide exchange factor son of sevenless (SOS) is recruited to activate the GTPase, which activates the MAPK kinase kinase RAF.

RAF phosphorylates the MAPK kinase MEK, which then phosphorylates MAPK. Phosphorylated MAPK becomes activated to perform its downstream actions. Two important MAPK effectors are the extracellular signal-regulated kinase (ERK) and the p38 MAPK, which promote mitosis and inflammation. This pathway is involved in the migratory ability of the cell, and its overactivation contributes to immunity (Ferguson et al., 2012).

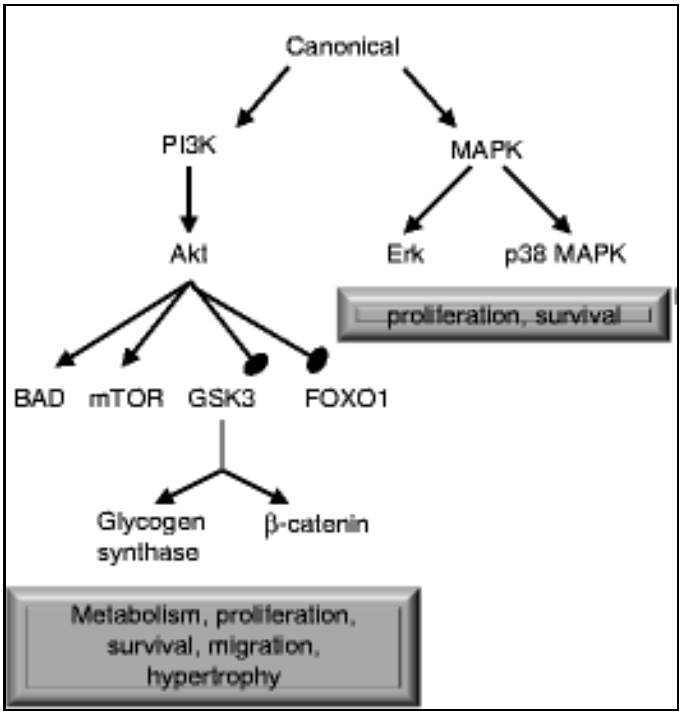

Figure 3. Overview of the downstream effects of IGF-IR. The binding of IGF-I to its receptor IGF-IR leads to two primary pathways—PI3K and MAPK. The diverse effects of IGF-IR activation include increased metabolism, proliferative ability, survival, migratory ability, and hypertrophy. Overactivation of these pathways is implicated in oncogenesis. Akt = protein kinase B; BAD = Bcl-2-associated death promoter; ERK = extracellular signal-regulated kinase; FOXO1 = forkhead box O1; GSK3 = glycogen synthase kinase 3; IGF-I = insulin-like growth factor-I; IGF-IR = insulin-like growth factor-I receptor; MAPK = mitogen-activated protein kinase; mTOR = mammalian target of rapamycin; PI3K = phosphoinositide 3-kinase. Figure modified from (Ferguson et al., 2012).

Non-small cell lung cancer is one of the disease states implicated with a dysregulated IGF-I pathway. In non-small cell lung cancers, miRNA-486 was found to be the most downregulated miRNA compared with miRNAs in other lung tissues (Peng et al., 2013). It was determined that the target of miRNA-486 was IGF-I. Since IGF activates separate pathways that affect cell survival and growth, overactivation of this growth factor, because of its loss of regulation by miRNA-486, could provide continuous stimulation of these proliferative pathways. Most important, when miRNA-486 was administered to non-small cell lung cancer cell lines, growth and migration were inhibited (Peng et al., 2013).

Evasion of Apoptotic Signals

The evasion of apoptosis allows tumors to progress without the normal signals that cause cell death upon the accumulation of mutations which could lead to oncogenesis. Proteins of the caspase family are the primary mediators of apoptosis, and they function as part of signaling cascades that amplify signals of apoptosis to lead to cell death. There are two pathways by which a cell can undergo apoptosis: (1) the intrinsic pathway or (2) the extrinsic pathway (Vaux, 2014). These two pathways are illustrated in **Figure 4** and are briefly summarized in the following description with details pertinent to this discussion.

1. In the intrinsic pathway, the mitochondria are primarily involved through the creation of the apoptosome. Cell damage and normal processes such as

developmental cues or lack of stimulation by growth factors can lead to activation of this pathway, which damages the mitochondria. The Bcl-2 family is the primary mediator of this pathway and consists of both pro-apoptotic and anti-apoptotic proteins. If the balance of these proteins is shifted in the apoptotic direction, then cytochrome c is released, and the apoptosome forms around the apoptotic protease-activating factor 1 (Apaf-1) protein. This process results in caspase-9 activation, which then activates caspase-3/7 and causes cell death (Vucic, 2014).

2. The death receptor (DR) family mediates signal transduction in the extrinsic pathway. This pathway is initiated when tumor necrosis factor (TNF) ligands bind tumor necrosis factor receptors (TNFRs). The binding of the ligands causes TNFRs to aggregate and recruit the death-inducing signaling complex (DISC). DRs and the first apoptosis signal (Fas) then associate with the Fas-associated protein with death domain (FADD) which activates caspases intracellularly. Caspase-8 activation triggers caspase-3/7 activation, leading to cell death. The TNFR recruits additional proteins to activate caspase-8 through the TNFR type 1-associated death domain (TRADD) and its accessory proteins [TNFR-associated factor 2 (TRAF2), c-inhibitor of apoptosis (c-IAP), and receptor-interacting protein 1 (RIP1)]. These proteins can also directly stimulate cell death by means of necroptotic pathways (Vucic, 2014).

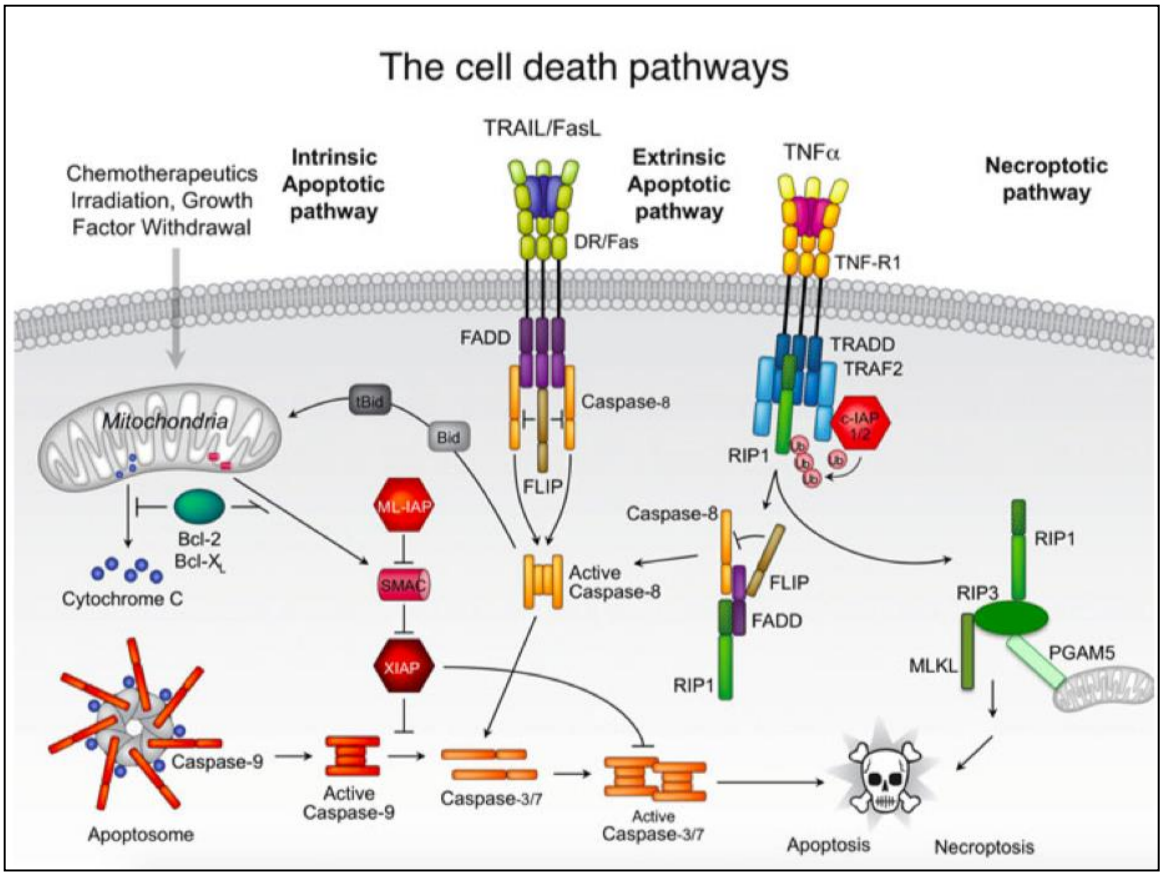

Figure 4. Intrinsic and extrinsic pathways of apoptosis. The intrinsic pathway involves mitochondrial release of cytochrome c, whereas the extrinsic pathway involves death receptors. Both pathways converge on the activation of caspase-3/7 to trigger apoptosis. Bcl = B-cell lymphoma; FADD = Fas-associated protein with death domain; Fas = first apoptosis signal; FLIP = FLICE-like inhibitory protein; IAP = inhibitor of apoptosis; MLKL = mixed lineage kinase domain-like; PGAM5 = phosphoglycerate mutase family member 5; RIP = receptor-interacting protein; TRADD = TNFR type 1-associated death domain; TRAF2 = TNFR-associated factor 2; TRAIL = TNF-related apoptosis-inducing ligand; TNF = tumor necrosis factor; TNF-R = tumor necrosis factor receptor (TNFR). Figure taken from (Vucic, 2014).

Apoptosis is partially controlled by p53, which is a tumor suppressor transcription factor that was described previously. There are two pathways that can be activated by p53

when DNA damage is sensed: (1) cell-cycle arrest and activation of DNA repair machinery or (2) apoptosis by either of the two pathways to prevent proliferation of potentially malignant damaged cells if the damage is unrepairable (Redondo, Fùnez, & Esteban, 2009). Thus, when the functions of p53 are antagonized, cancer is able to progress beyond the control mechanisms of programmed cell death. One such way of accomplishing this is with murine double minute 2 (MDM2), a protein that degrades p53. As a transcription factor, p53 induces the production of certain miRNAs which are responsible for the tumor-suppressing effects of p53. In a study of multiple myeloma, a B-cell neoplasm, it was found that miRNA-192, miRNA-194, and miRNA-215, which function to suppress the expression of MDM2, were epigenetically downregulated. This leads to low levels of p53 and subsequent suppression of its anti-apoptotic functions (Pichiorri et al., 2010).

Another important regulator of apoptosis is the Bcl-2 protein family. Bcl-2 is an anti-apoptotic factor that is often overexpressed in cancer and is critically involved in the intrinsic pathway of apoptosis. For example, in the previously discussed case of CLL, which is caused by a deletion in miRNA-15 and miRNA-16, it was found that the mRNA for Bcl-2 is a target for these miRNAs (Cimmino et al., 2005). Thus, with the low levels of these miRNAs in CLL, cancerous cells are able to evade apoptosis because of the lack of negative regulation on the anti-apoptotic factor Bcl-2. Most important, when miRNA-15 and miRNA-16 were re-introduced into the CLL cell line, they caused apoptosis by means of Bcl-2 repression (Cimmino et al., 2005). Similarly, miRNA-365 (Nie et al.,

2012), miRNA-204 (Sacconi et al., 2012), and miRNA-148a (H. Zhang et al., 2011) have all been found to regulate Bcl-2 in this way.

Tissue Invasion and Metastasis

The action of epithelial-to-mesenchymal transition (EMT) is a normal process involved in fetal development and wound healing. In this process, epithelial cells lose their lateral adhesion and are able to move and transform into mesenchymal cells. However, the same mechanisms involved with this process are implicated in malignant cancer progression and metastasis (Roche, 2018). In addition, the EMT process is in a transitional state in cancer, possessing both epithelial and mesenchymal phenotypes. This condition gives cancerous cells more aggressive capabilities, such as the ability to move in clusters. In order for EMT to occur, there must be disruption of cell-cell adhesion (mediated by E-cadherin), cell-matrix adhesion, normal cell polarity, and the cell cytoskeleton. The genetic transition from epithelial to mesenchymal is triggered by the following transcriptional regulators: Snail1 (SNAI1), SNAI2, zinc-finger E-box-binding homeobox 1 (ZEB1), ZEB2, Twist, and E12/E47. The overall process is controlled by transforming growth factor-β (TGF-β), a multifunctional cytokine. Additional regulators include hypoxia, growth factors, metabolic changes, and immune response (Roche, 2018).

The miRNA-200 family consists of five members: miRNA-200a, miRNA-200b, miRNA-200c, miRNA-141, and miRNA-429. It was found that the miRNA-200 family and miRNA-205 were underexpressed in cells during the process of EMT in which TGF-

β was used as the initiator (Gregory et al., 2008). In addition, enforced expression of the miRNA-200 family inhibited EMT. The mechanism by which these miRNAs prevent EMT is through the suppression of the transcription repressors for E-cadherin. If the transcription repressors are silenced, E-cadherin continues to be produced. As a result, the cell does not undergo EMT but can actually undergo the reverse process—mesenchymal to epithelial transition (MET). The observation that these miRNAs were lost in breast cancer could explain the progression of tumors in these cases (Gregory et al., 2008).

The transcription factor p53 has also been shown to play a role in EMT through the direct activation of miRNA-200c. When p53 is downregulated in cancer, this leads to reduced production of miRNA-200c and a subsequent increase in stem cell production (Chang et al., 2011). Furthermore, p53 plays a role in repressing the expression of ZEB1 and ZEB2, transcription factors that promote EMT. This is accomplished by means of the p53-mediated upregulation of miRNAs that repress ZEB1/2 expression (miRNA-200 and miRNA-192 families) (Kim et al., 2011).

The downregulation of two other important miRNAs (miRNA-203 and miRNA-212) is implicated in promoting EMT and giving tumorigenic cells metastatic or invasive potential. In highly metastatic breast cancer cells, it was determined that miRNA-203 was epigenetically downregulated (Ding, Park, McCauley, & Wang, 2013). There is a reciprocal repressive or double-negative feedback mechanism between SNAI2 and miRNA-203. This balance is shifted toward SNAI2 when TGF-β, a factor that promotes EMT, further induces SNAI2 to repress miRNA-203. If miRNA-203 is replete, SNAI2 is

repressed, yielding the inhibition of invasive or metastatic potential (Ding et al., 2013). Likewise, miRNA-212 has been studied in colorectal cancer (Meng et al., 2013). The compound manganese superoxide dismutase was involved in promoting factors that support EMT, which is downregulated by miRNA-212 under physiological conditions. However, downregulation of miRNA-212 by genetic or epigenetic mechanisms allowed for the expression of manganese superoxide dismutase in colorectal cancer, leading to more aggressive tumors and a worse prognosis in patients. When miRNA-212 was restored, the EMT process was blocked (Meng et al., 2013).

Inducing Sustained Angiogenesis

Cancer is inherently linked to increased vascularity to provide oxygen to the growing tumor cells. Angiogenesis is an essential process that occurs during embryogenesis and is controlled by diverse factors. Of central importance in regulation of this process is vascular endothelial growth factor (VEGF). The initiation of growth and the maturation of new vessels depend primarily on the signaling induced by this growth factor. VEGF can also induce the production of the anti-apoptotic protein Bcl-2, and in this way it can prevent the death of cells in hypoxic conditions, in addition to producing new blood vessels to relieve hypoxic conditions. It would follow that levels of oxygen are the primary regulator of VEGF expression. This is accomplished through hypoxia-inducible factor 1α (HIF-1α), which is released under hypoxic conditions (Ferrara, 2008).

There are three known miRNAs that serve to protect against physiological tumor angiogenesis: miRNA-20b, miRNA-519c, and miRNA-107. miRNA-20b is regulated by

conditions of oxygen saturation. When miRNA-20b is inhibited, the protein levels of HIF-1α and VEGF increase. Because a reciprocal regulation mechanism exists in this system as well, the expression of HIF-1α directly inhibits miRNA-20b (Lei et al., 2009). HIF-1α is also regulated by miRNA-519c through the direct binding of the miRNA onto HIF-1α mRNA. If miRNA-519c is overexpressed, there is a significant decrease in HIF-1α and a subsequent reduction in tumor angiogenesis (Cha et al., 2010). Finally, miRNA-107 is involved in preventing angiogenesis through a role mediated by p53. As a transcription factor, p53 upregulates miRNA-107 to decrease hypoxia signaling. In colon cancer, it was found that miRNA-107 suppresses HIF-1β, and overexpression of miRNA-107 can suppress tumor angiogenesis and VEGF expression (Yamakuchi et al., 2010). In summary, downregulation of any of these miRNAs can contribute to the potential for angiogenesis in tumorigenic cells, and when overexpressed, these miRNAs can be used as potent inhibitors of tumor progression.

REPLETION OF DOWNREGULATED miRNAs IN CANCER MODELS

This section provides an overview of current efforts in preclinical models for miRNA replacement therapy. Although the studies presented in this section refer to targeting a specific gene, the regulation and downstream effects of miRNAs are diverse because of the fact that each miRNA regulates many genes. In addition, there is a large genetic diversity in tumor cells as previously discussed. This is further complicated by the fact that there is a considerable amount of redundancy within miRNAs and their targets. Families of miRNAs often have redundant functionality; thus, there is often no loss of function unless a whole family of miRNAs is downregulated (Alvarez-Saavedra & Horvitz, 2010). Different miRNAs can also target and repress different sections of the same mRNA (Tsang, Ebert, & van Oudenaarden, 2010). Finally, single miRNAs can target many genes, and when any one miRNA is downregulated, there are diverse implications for the entire system.

Potential Target miRNAs Downregulated by Cancer

Based on miRNA expression profiles in cancers studied by Lu and colleagues (2005), downregulation of distinct miRNAs has been linked to various cancers. The top ten miRNAs downregulated in specific cancers are compiled in **Table 1.**

Table 1. Downregulated miRNAs Linked to Cancers by System[a]

Breast (T/N)[b]	Lung (T/N)	Kidney (T/N)	Colon (T/N)	Prostate (T/N)	Uterus (T/N)	Breast (PDT/T)[c]	Lung (PDT/T)	Ovary (PDT/T)
miR-199b	miR-1	miR-194	miR-1	miR-1	miR-1	miR-29c	miR-29c	miR-33
miR-99a	miR-33	miR-215	miR-213	miR-33	miR-126	miR-33	miR-221	miR-210
miR-130a	miR-99a	miR-124a	miR-195	miR-144	miR-195	miR-221	miR-182	miR-15a
miR-100	miR-126	miR-204	miR-125b	miR-213	miR-140	miR-15a	miR-214	miR-150
miR-150	miR-144	miR-22	miR-96	miR-206	miR-213	miR-152	miR-148b	miR-148b
miR-195	miR-195	miR-199a	miR-99a	miR-150	miR-154	miR-148b	miR-181b	miR-181a
let-7b	miR-154	miR-199a	let-7a	miR-142-3p	miR-182	miR-193	miR-199a	miR-149
miR-154	miR-196a	miR-186	miR-30b	miR-24	miR-214	miR-214	miR-181a	
miR-196a	miR-130a	miR-191	miR-153	miR-126	miR-10b	miR-29a	miR-15a	
miR-126	miR-152	miR-99a	miR-19b	miR-199b	miR-150	miR-187	miR-187	

[a]Summary of the top 10 miRNAs that are downregulated in cancers classified by their system. Poorly differentiated ovarian tumor has only 7 downregulated miRNAs of significance. let = lethal; miR = microRNA; miRNA = microRNA. Based on data collected by (Lu et al., 2005) and analyzed and compiled by (Cohen, Burgos-Aceves, & Smith, 2018).
[b]T/N: tumor versus normal tissue.
[c]PDT/T: poorly differentiated tumor (tumors that are more abnormal; i.e., tumors that have less features similar to normal tissue of the system and are associated with higher tumor grades) versus tumor.

Although the expression of these miRNAs has been shown to be downregulated in these cancers (**Table 1**), repletion of any miRNA in particular is not certain to reverse or inhibit the oncogenic phenotype. This is due to differing endogenous effects, which have not been fully elucidated for all of the listed miRNAs. Therefore, studies have focused on repletion of miRNA expression for those candidates that have been studied and proved to support a tumor-suppressive function. In particular, some miRNAs have been identified as being master regulators of a variety of tumor-suppressive functions. In these cases, multiple oncogenic processes can be impeded by the repletion of a single miRNA or miRNA family. Many of these studies have shown potential to slow or reverse the processes involved with oncogenesis in vivo or in tumor cell models.

Overview of Preclinical Models for miRNA Replacement Therapy

From the discovery of miRNA in 1993 (Lee, Feinbaum, & Ambros, 1993), the field of research in miRNA has blossomed. Over 2500 human miRNAs have been identified (Kozomara & Griffiths-Jones, 2011), and the downregulation of many of these miRNAs has been linked to cancer. **Table 2** presents an overview of candidate miRNAs that have demonstrated anticancer effects in preclinical models. In all of these cases, the miRNA was identified as being downregulated in the system under investigation. The studies referenced in **Table 2** were performed either in vivo or in a cell culture model, with the miRNA listed in the first column being used by different experimental methods (some of which are described in the next section) to replenish the downregulated miRNA in that system. Thus, these studies provide important foundations for future developments

in miRNA replacement therapy as it moves toward trials in nonhuman primates and humans and eventually toward use as a molecular targeted cancer therapy in the clinic.

Table 2 was partially compiled from the work of Nayer, Mahyar, Duijf, and Behzad (2018) and supplemented with 73 additional studies to provide a more thorough survey of ongoing efforts in this field. Nevertheless, even with these additions, this table is not a comprehensive survey of all the systems or tissues in which miRNA replacement has been evaluated. Rather, the intention of this table is to provide a general overview of candidate miRNAs that have been studied to date with examples from selected systems. Although the main targets that were found to be repressed in these studies are indicated, it is important to note that the miRNAs have a wide range of other targets as well. However, each specified target has a demonstrated link to oncogenesis involving one of the mechanisms described in the previous sections. The studies referenced in **Table 2** have been ordered numerically and compiled independently in the **Appendix**.

Table 2. Survey of Preclinical Studies in miRNA Replacement Therapy[a]

miRNA	**System/Tissue**	**Target**	**Study**
Let-7	Liver	C-myc, p16	1
	Lung	KRAS	2
	Colon	RAS, C-myc	3

Table 2 (continued). Survey of Preclinical Studies in miRNA Replacement Therapy[a]

miRNA	System/Tissue	Target	Study
Let-7	Laryngeal	RAS, C-myc	4
	Gastric	PKM2	5
Let-7, 34a	Lung	KRAS, P53	6, 7
7	Adrenal	Raf-1	8
	CNS	OGT	9
	CNS	EGFR	10
10b	Uterine	HOXB3	11
16	Lung	VEGF	12
	Prostate	CDK1, CDK2	13
26a	Breast	MTDH	14
27a	Breast	MDR-1	15
29	Liver	Bcl-2, Mcl-1	16
29b	Blood	SP1, CDK6, KIT	17
	Blood	HDAC	18
34a	Lung	Bcl-2	19, 20
	Pancreas	Bcl-2	21
	Gastric	Bcl-2	22
	CNS	Bcl-2, MMP2	23

Table 2 (continued). Survey of Preclinical Studies in miRNA Replacement Therapy[a]

miRNA	System/Tissue	Target	Study
	Colon	E2F, P53	24
34a	Prostate	Bcl-2, CD44, SIRT1	25
	Melanoma	MAPK	26
	Bone	Notch1/ROCK1-PTEN-Akt-GSK-3β	27
	Breast	SIRT1	28
	Liver	C-myc	29
99a	Renal	mTOR	30
100	Bladder	mTOR	31
101	Liver	Mcl-1	32
	Liver	NLK, EZH2, Mcl-1, STMN1, Rab5A	33
	Liver	EZH2, COX2, STMN1, ROCK2	34
107	Head & neck	PKCε, CDK6, HIF1-β, Sox2	35
122	Liver	Bcl-2	36
	Breast	IGF-1R	37
	Liver	ADAM10, SRF, IGF-1R	38
124	Colon	PTB1	39
125a-5p	Breast	HER2	40
	Gastric	ERBB2	41

Table 2 (continued). Survey of Preclinical Studies in miRNA Replacement Therapy[a]

miRNA	System/Tissue	Target	Study
125a-5p	Colon	Bcl-2, Bcl-2L12, Mcl-1	42
	CNS	TAZ	43
	Prostate	NAIF1, Caspase 3	44
126	Gastric	Crk	45
128	Prostate	BMI-1	46
133b	Gastric	FBN1	47
133-b	Lung	Mcl-1	48
135a	CNS	STAT6, SMAD5, BMPR2	49
137, 124	CNS	CDK-6	50
143	Bone	MMP13	51
143, 145	Colon	ERK-5	52
144	Liver	EGFR, Src/Akt	53
145, 33a	Colon	C-myc, ERK-5, Pim-1	54
145	Colon	STAT-1, YES	55
	Lung, CNS	Oct4, Sox2	56
	Gastric	Ets1	57
	Lung	Oct4/Sox2/Fascin1, Tcf4, Wnt5a	58
	Colon	p70S6K1 (VEGF/HIF-1), IRS-1	59, 60

Table 2 (continued). Survey of Preclinical Studies in miRNA Replacement Therapy[a]

miRNA	System/Tissue	Target	Study
145	Liver	IRS-1, IRS-2, IGF-1R	61
	Breast	Fascin-1, C-myc, SMAD2/3, IGF-1R, ER-α	62, 63
146a	Liver	EGFR, ERK1/2, STAT5	64
146b	CNS	EGFR, NF-κB, SMAD4	65
148a	Breast	Bcl-2	66
148a, 152	Breast	IGF-IR, IRS1	67
150	Pancreas	MUC4	68
182	CNS	Bcl-2L12, c-Met, HIF2-α	69
185	Liver	RHEB, RICTOR, AKT1	70
185-3p, 324-3p	Nasopharyngeal	SMAD7	71
193a-3p	Lung	KRAS	72
187	Colon	CD276	73
195	Liver	ROCK2, VEGF	74
200c	Lung	ZEB1	75
	Liver	ZEB1, ZEB2	76
	Ovarian	TUBB3	77
200b/c	Liver	SUZ12/ROCK2	78

Table 2 (continued). Survey of Preclinical Studies in miRNA Replacement Therapy[a]

miRNA	System/Tissue	Target	Study
203	Breast	SNAI2	79
203	Bladder	Bcl-2	80
206	Liver	cMET, Cyclin D1, CDK6	81
302	Uterine	Cyclin D1, CDK1	82
320	Lung	Bcl-2, Bax, Caspase 3	83
326	Breast	MRP-1/ABCC1	84
340	Liver	JAK1/STAT3	85
372	Head & Neck	p62	86
375	Liver	AEG-1, ATG7	87
	Esophageal	LDHB, MTDH	88
	Head & Neck	LDHB	89
378	Gastric	MAPK-1	90
382	Lung	SETD8	91
424	Blood	BCR-ABL	92
449	Prostate	Cyclin D1, HDAC1	93
451	Breast	MDR-1	94
451	CNS	Bcl-2, MMP-2, MMP-9, Akt1, Cyclin D1	95
486	Lung	IGF-1, IGF-1R, PIK3R1	96

Table 2 (continued). Survey of Preclinical Studies in miRNA Replacement Therapy[a]

miRNA	System/Tissue	Target	Study
491	Ovarian	Bcl-XL, EGFR	97
495	Colon	FAM83D	98
508-5p	CNS	GPNMB	99
520h	Pancreas	ABCG2	100
542-3p	Breast	p53, Survivin	101
603	Breast	EF2K	102
885-3p	Colon	Smad1/5/8, ID-1	103
1258	Breast	Heparanase	104

[a]Partially compiled with studies from (Nayer, Mahyar, Duijf, & Behzad, 2018) and supplemented with 73 additional studies (Appendix) to provide a more complete overview of current efforts in the development of miRNA replacement therapy. CNS = central nervous system; miRNA = microRNA.

DEVELOPMENT OF miRNA THERAPEUTICS IN CANCER

As presented in the previous section, there are a great diversity of candidates and encouraging results from preclinical studies performed in miRNA replacement. However, the many barriers toward translation of these encouraging preclinical results to the clinic have led to a slowing down of further development in this field. To date, there are only two completed phase I studies that have attempted to administer miRNA replacement therapeutics in an oncologic clinical trial (Beg et al., 2017; van Zandwijk et al., 2017). This section discusses the major hurdles in the pharmacological development of miRNA replacement therapies. It also evaluates the published results of the past two clinical trials and discusses the factors that have contributed to the lack of positive results.

Challenges and Strategies in the Use of miRNAs as Therapeutic Agents

Successful delivery of miRNAs to cancerous tissue is one of the greatest hurdles toward the clinical application of miRNA replacement therapy. As illustrated in **Figure 5**, miRNA replacement therapy can be divided into two subcategories of strategies: the use of miRNA mimics and the use of DNA plasmids to express miRNAs. Of the in vivo studies previously described, delivery was most often accomplished by viral vectors, liposomal transfection, or direct injection into the tumor site. Intratumoral injections are not widely practical in the clinic because of the small fraction of cases in which the tumor site is easily accessible and not metastasized. Therefore, the delivery options with the

best potential for translation to the clinic are lipid vesicles, dendrimers, gold nanoparticles, polymeric nanoparticles, and viral vectors.

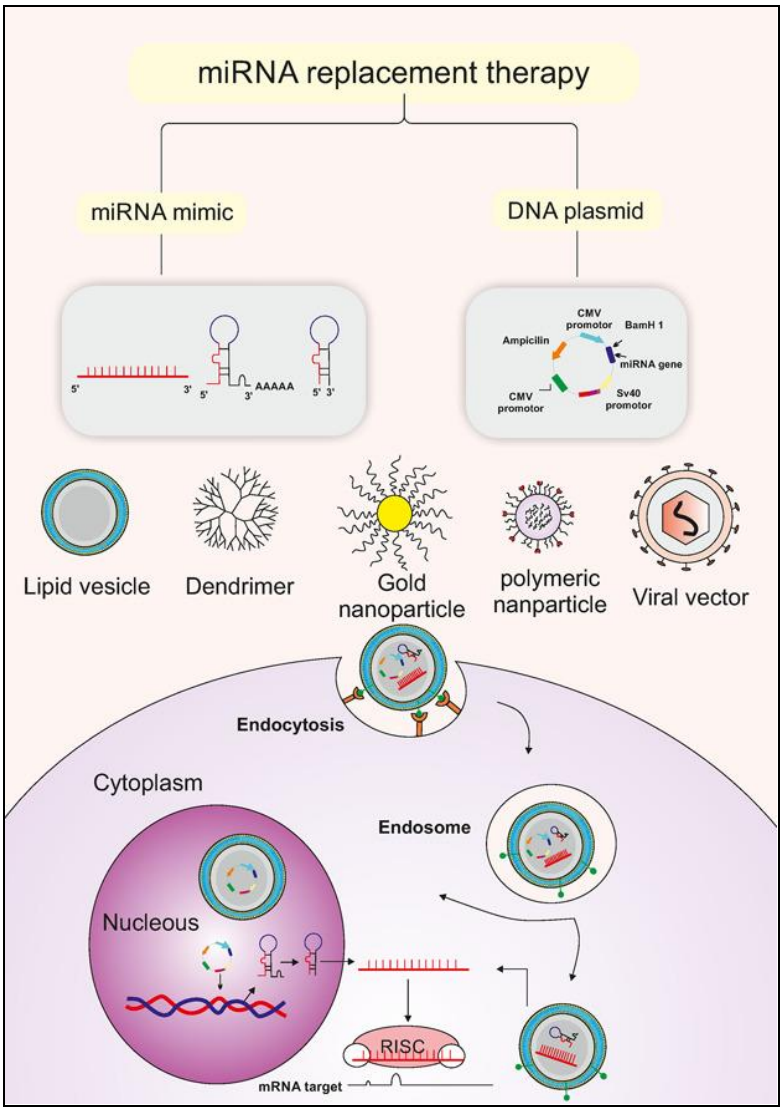

Figure 5. Strategies in miRNA replacement therapy. miRNA replacement therapy is executed through the use of miRNA mimics or the use of DNA plasmids that express miRNA. There are five broad categories for delivery methods: lipid vesicles, dendrimers, gold nanoparticles, polymeric nanoparticles, and viral vectors. BamH 1 = Bacillus amyloliquefaciens H 1; CMV = cytomegalovirus; miRNA = microRNA; mRNA = messenger RNA; RISC = RNA-induced silencing complex; Sv40 = simian virus 40. Figure taken from (Hosseinahli, Aghapour, Duijf, & Baradaran, 2018).

Viral vectors have numerous issues preventing their successful use, including poor infectivity of target tissue, and these hurdles have been subjected to extensive research in the field of gene therapy. In addition, viral vectors generally involve a DNA vector and endogenous processing for maturation of the final product. Because many miRNA biogenic processes are impaired in cancer, this method of miRNA delivery is not likely to lead to successful clinical application (Trang et al., 2011). Thus, of the remaining nonviral methods of delivery, lipid vesicles and polymeric nanoparticles are the most important to examine

Endogenous extracellular or circulating miRNA is stable in peripheral blood. Although, in general, RNA is very unstable and subject to quick degradation by nucleases, miRNAs are immune to this to a certain extent. This is because the miRNAs travel in exosomes or microvesicles and associate with Argonaute proteins. It has been hypothesized that their stability is due to the fact that the circulating miRNAs are contained in the lipid membrane of dead cells (Turchinovich, Weiz, Langheinz, & Burwinkel, 2011). In an attempt to mimic this endogenous stability of miRNAs in plasma, the use of lipid vesicles is among the most popular methods of introducing miRNA into systems in preclinical models.

The miRNA-based therapeutic MRX34, an miRNA-34 mimic delivered in a liposomal nanoparticle, deserves special recognition as the first in its class to enter clinical testing. MRX34 uses patented nanoparticles in a technology given the trade name SMARTICLES® (Kelnar, Peltier, Leatherbury, Stoudemire, & Bader, 2014). This delivery vesicle consists of four types of lipids, some found endogenously in eukaryotic

cell membranes, with differing acidic and basic characteristics. The liposome is cationic during manufacturing to stabilize the oligonucleotide, but it can modify its pH physiologically to become anionic in the optimal range to facilitate entry to the target cell membranes (Tolcher et al., 2014). In animal models of liver tumors, this liposomal systemic delivery was found to cause regression of the cancer with no activation of immune response or toxic side effects (Daige et al., 2014). Thus, this delivery method showed great promise, and the FDA approved this investigational new drug for testing in humans.

Another method that showed success in preclinical models is targeted bacterial-derived nanoparticles. This alternate delivery method was investigated because of certain key limitations in liposome drug delivery; namely, drug leakage and reduced potency. The pharmacological agent is able to be encapsulated in these nanoparticles, called minicells, which are derived from intentionally induced improper bacterial cell division. Furthermore, these minicells can be targeted to the site of interest through conjugation of the outer surface with antibodies (MacDiarmid et al., 2007). For example, antibodies to the epidermal growth factor receptor (EGFR) can be used to target the delivery to tumor cells because these receptors are overexpressed in cancer (Nicholson, Gee, & Harper, 2001). This method, with the trade name EnGeneIC Dream Vector (EDV)^{TM} nanocells, was used to deliver an miRNA-16 mimic to an animal model of malignant pleural mesothelioma. The results of this study showed tumor regression and specific targeting to cancerous cells but not normal mesothelial cells (Reid et al., 2013). Based on the favorable results of this study, a phase I clinical trial was initiated.

Clinical Trials in miRNA Replacement in Cancer

The two clinical trials initiated in miRNA cancer therapeutics use miRNA mimics for repletion of two key candidates, miRNA-34a and miRNA-15/16. Both of these miRNA families are master regulators of tumor suppressor activity. They serve to promote a wide range of anti-oncogenic functions through the targeting of diverse genes. If these genes are overexpressed, they contribute to oncogenesis by multiple mechanisms (**Figure 6**). The efficacy and safety of these clinical trials are discussed in this section.

MRX34: A Mimic of miRNA-34

In many cancers, it has been found that miRNA-34 is downregulated. As discussed previously, miRNA-34 shares a regulatory axis with the tumor suppressor p53 (Chang, Wentzel, et al., 2007) and is known to control more than 30 oncogenes (some of which are listed in **Figure 6**). A phase I clinical trial was conducted on patients with advanced solid tumors refractory to standard treatment (Beg et al., 2017). Over the 17 weeks of the trial, 47 patients were given at least one dose of the medication, and over 60% of the treated patients discontinued the study because of disease progression. Of the 41 patients who were able to be evaluated for tumor progression, one patient partially responded to the treatment, 6 patients were stabilized, and 32 patients progressed in their disease. Infusion-related adverse effects were a significant concern for this study, as they interfered with the administration of the medication and thus did not allow for continuous and consistent dosing. Although direct cause could not be determined, it was postulated that the infusion-related adverse effects were related to the liposomal delivery method. The authors attributed the majority of the adverse effects to general immune reactions

that occur in response to dsRNA. Although there was response in some patients, this study was terminated prematurely because of the number of adverse events that were classified as serious (Beg et al., 2017).

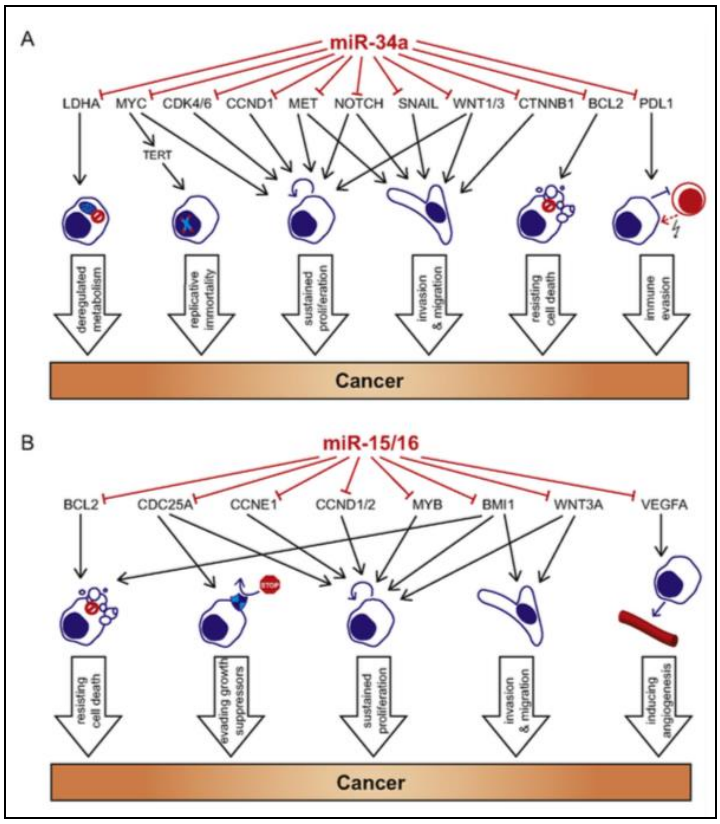

Figure 6. Molecular targets of miRNAs in clinical development. (A) miRNA-34 and **(B)** miRNA-15/16 serve tumor suppressor functions in many oncogenic pathways, and they are significantly downregulated in many cancers. This figure provides examples of the oncogenes and the resulting tumorigenic properties that these miRNAs inhibit. BCL = B-cell lymphoma; BMI1 = B-cell-specific Mo-MLV insertion site-1; CDC25A = cell division cycle 25A; CDK = cyclin-dependent kinase; LDHA = lactate dehydrogenase A; miR = microRNA; miRNA = microRNA; PDL = programmed death ligand; TERT = telomerase reverse transcriptase; VEGF = vascular endothelial growth factor. Figure modified from (Ivkovic, Voss, Cornella, & Ceder, 2017).

TargomiRs: A Mimic of miRNA-16

The loss of miRNA-15/16 is noted in many human malignancies. After promising results in the preclinical use of minicells with miRNA-16 mimics targeted to EGFR to treat mesothelioma (Reid et al., 2013), a clinical trial was conducted with the same therapy. In this phase I study, 27 patients with malignant pleural mesothelioma and tumors significantly expressing EGFR were enrolled (van Zandwijk et al., 2017). More than half of the patients reported the adverse event of non-cardiac chest pain that the investigators believed was related to accumulation of the drug around the leaky tumor vasculature. Nevertheless, the investigators found significant stabilization of disease progression and even a partial response that lasted for 32 weeks in one patient. These results are encouraging for future trials, but no significant conclusions can be drawn because of the small sample size. In addition, the maximum tolerated dose was quite low compared with the minimum effective dose that was found in murine studies. The investigators postulated that the serious inflammation-related adverse events, which caused only a low dose to be tolerated, were due to the minicell carrier method and not the miRNA mimic. These results encourage future development with this candidate, possibly with different routes of administration or delivery methods, and phase II trials are likely to take place with some modifications to the candidate (van Zandwijk et al., 2017).

FUTURE DIRECTIONS AND CONCLUSIONS

The Potential for miRNA in Gene Silencing

Treating disease through knockdown of specific genes is an attractive concept that has been under speculation with the discovery of RNA interference. Accomplishing knockdown by the use of synthetically developed miRNA is one technique to achieve this goal. This concept is beginning to be investigated in cancer. For example, one study aimed to downregulate heparanase, an enzyme involved in promoting metastasis, by the application of an artificially created miRNA (Liu et al., 2012). The investigators designed an miRNA based on miRNA-155 and modified the key sequence to target heparanase. The artificial miRNA showed effective inhibition of heparanase mRNA and knockdown of the resulting protein, decreasing metastasis both in vitro and in vivo. In addition, an important benefit of this method is that tissue-specific expression can be achieved using special RNA polymerase II promoters (Liu et al., 2012).

Although studies such as the one by X. Liu and colleagues (2012) showed proof of concept, it is not clear whether this method of RNA interference will be the subject of future development. The artificial miRNA modified from miRNA-155 has shown promise in targeted knockdown of one enzyme in cancerous tissue, and its use could theoretically provide the potential benefit of being able to target and knock down many different oncogenic genes with one sequence. However, in practical use, this benefit versus the benefits of other gene therapy methods has not yet been established.

Furthermore, translating this method to clinical use will face the same setbacks as the rest of the field of gene therapy because of the lack of development of well-tolerated and efficacious delivery systems.

Conclusions

There is currently an unmet need in targeted cancer therapies. However, with better understanding of the genome of cancers, the molecular mechanisms of oncogenesis, and gene therapy, much progress is being made in this arena. The discovery of the link between cancer and miRNA has fueled a new field of research and development. Specifically, progress has been made in uncovering how mechanisms of oncogenesis can lead to changes in the regulation of miRNA and how the downregulation of miRNA contributes to the formation of cancer. These reciprocal mechanisms are emerging as a hallmark of cancer and provide an excellent target for therapy.

The use of miRNAs as therapeutic agents has garnered considerable interest as techniques in gene therapy become more refined. The concept of repletion of miRNA expression when it is downregulated in cancer has shown much progress in preclinical studies. miRNA replacement therapy is an attractive concept because it attempts to treat cancer through restoration of an endogenous pathway that has gone awry, and as a result, it may possibly lead to less toxic side effects than more traditional cancer-killing therapeutic concepts.

This thesis has reviewed the mechanisms underlying miRNA and its links to cancer and provided an overview of in vitro and in vivo studies working to remedy the

imbalance caused by oncogenic processes. In particular, this thesis has given examples of over 100 studies in which miRNA replacement therapy has exhibited extremely efficacious results in preclinical models of cancer. There are numerous examples of candidate miRNAs that show the potential to silence many different targets in diverse systems of cancer. Unfortunately, with only two clinical trials completed in this field, these successes at the bench have not yet been translated into therapeutic successes in patients. Nevertheless, there are several clinical trials in progress in cancer and other diseases.

The greatest impediment to the clinical use of miRNA is the availability of effective delivery systems. However, incredibly diverse techniques for nonviral delivery of pharmacological agents are beginning to be introduced. The promising results seen in the TargomiRs trial, which used nanoparticles to directly target the cancer site, provide hope for the future, especially with the continued development in phase II clinical trials. With a small amount of refinement, miRNA therapeutic agents hold boundless potential as a safer and more efficacious treatment in cancer.

APPENDIX

REFERENCES FOR TABLE 2

1. Lan Fei-Fei, Wang Hua, Chen Yang-Chao, et al. Hsa-let-7g inhibits proliferation of hepatocellular carcinoma cells by downregulation of c-Myc and upregulation of p16INK4A. *International Journal of Cancer*. 2010;128(2):319-331. doi:10.1002/ijc.25336

2. Trang P, Medina PP, Wiggins JF, et al. Regression of murine lung tumors by the let-7 microRNA. *Oncogene*. 2009;29:1580. doi:10.1038/onc.2009.445

3. Akao Y, Nakagawa Y, Naoe T. let-7 MicroRNA functions as a potential growth suppressor in human colon cancer cells. *Biological and Pharmaceutical Bulletin*. 2006;29(5):903-906. doi:10.1248/bpb.29.903

4. Liu. Let-7a microRNA functions as a potential tumor suppressor in human laryngeal cancer. *Oncology Reports*. 2009;22(05). doi:10.3892/or_00000554

5. Tang R, Yang C, Ma X, et al. MiR-let-7a inhibits cell proliferation, migration, and invasion by down-regulating PKM2 in gastric cancer. *Oncotarget*. 2016;7(5). doi:10.18632/oncotarget.6821

6. Stahlhut C, Slack FJ. Combinatorial action of microRNAs let-7 and miR-34 effectively synergizes with erlotinib to suppress non-small cell lung cancer cell proliferation. *Cell Cycle*. 2015;14(13):2171-2180. doi:10.1080/15384101.2014.1003008

7. Wiggins JF, Ruffino L, Kelnar K, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer Research.* 2010;70(14):5923. doi:10.1158/0008-5472.CAN-10-0655

8. Glover AR, Zhao JT, Gill AJ, et al. microRNA-7 as a tumor suppressor and novel therapeutic for adrenocortical carcinoma. *Oncotarget*. 2015;6(34):36675-36688. doi:10.18632/oncotarget.2235

9. Babae N, Bourajjaj M, Liu Y, et al. Systemic miRNA-7 delivery inhibits tumor angiogenesis and growth in murine xenograft glioblastoma. *Oncotarget*. 2014;5(16):6687-6700. doi:10.18632/oncotarget.2235

10. Liu X, Li G, Su Z, et al. Poly(amido amine) is an ideal carrier of miR-7 for enhancing gene silencing effects on the EGFR pathway in U251 glioma cells. *Oncology Reports*. 2013;29(4):1387-1394. doi:10.3892/or.2013.2283

11. Chen H, Fan Y, Xu W, et al. miR-10b inhibits apoptosis and promotes proliferation and invasion of endometrial cancer cells via targeting HOXB3. *Cancer Biotherapy and Radiopharmaceuticals*. 2016;31(6):225-231. doi:10.1089/cbr.2016.1998

12. Tung Y-T, Huang P-W, Chou Y-C, et al. Lung tumorigenesis induced by human vascular endothelial growth factor (hVEGF)-A(165) overexpression in transgenic mice and amelioration of tumor formation by miR-16. *Oncotarget*. 2015;6(12):10222-10238. doi:10.18632/oncotarget.3390

13. Takeshita F, Patrawala L, Osaki M, et al. Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. *Molecular Therapy*. 2010;18(1):181-187. doi:10.1038/mt.2009.207

14. Liu P, Tang H, Chen B, et al. miR-26a suppresses tumour proliferation and metastasis by targeting metadherin in triple negative breast cancer. *Cancer Letters*. 2015;357(1):384-392. doi:10.1016/j.canlet.2014.11.050

15. Zhu H, Wu H, Liu X, et al. Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. *Biochemical pharmacology*. 2008;76(5):582-588. doi:10.1016/j.bcp.2008.06.007

16. Xiong Yujuan, Fang Jian-Hong, Yun Jing-Ping, et al. Effects of MicroRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. *Hepatology*. 2009;51(3):836-845. doi:10.1002/hep.23380

17. Huang X, Schwind S, Yu B, et al. Targeted Delivery of microRNA-29b by Transferrin conjugated anionic lipopolyplex nanoparticles: A novel therapeutic strategy in acute myeloid leukemia. *Clinical cancer research: an official journal*

of the American Association for Cancer Research. 2013;19(9):2355-2367. doi:10.1158/1078-0432.CCR-12-3191

18. Amodio N, Stamato MA, Gullà AM, et al. Therapeutic targeting of miR-29b/HDAC4 epigenetic loop in multiple myeloma. *Molecular Cancer Therapeutics*. 2016;15(6):1364. doi:10.1158/1535-7163.MCT-15-0985

19. Trang P, Wiggins JF, Daige CL, et al. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. *Molecular Therapy*. 2011;19(6):1116-1122. doi:10.1038/mt.2011.48

20. Wiggins JF, Ruffino L, Kelnar K, et al. Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer research*. 2010;70(14):5923-5930. doi:10.1158/0008-5472.CAN-10-0655

21. Ji Q, Hao X, Zhang M, et al. MicroRNA miR-34 Inhibits human pancreatic cancer tumor-initiating cells. Bernhard EJ, ed. *PLoS ONE*. 2009;4(8):e6816. doi:10.1371/journal.pone.0006816

22. Ji Q, Hao X, Meng Y, et al. Restoration of tumor suppressor miR-34 inhibits human p53-mutant gastric cancer tumorspheres. *BMC Cancer*. 2008;8:266-266. doi:10.1186/1471-2407-8-266

23. Tivnan A, Orr WS, Gubala V, et al. Inhibition of neuroblastoma tumor growth by targeted delivery of microRNA-34a using anti-disialoganglioside GD(2) coated nanoparticles. Chesler L, ed. *PLoS ONE*. 2012;7(5):e38129. doi:10.1371/journal.pone.0038129

24. Tazawa H, Tsuchiya N, Izumiya M, Nakagama H. Tumor-suppressive miR-34a induces senescence-like growth arrest through modulation of the E2F pathway in human colon cancer cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104(39):15472-15477. doi:10.1073/pnas.0707351104

25. Liu C, Kelnar K, Liu B, et al. Identification of miR-34a as a potent inhibitor of prostate cancer progenitor cells and metastasis by directly repressing CD44. *Nature medicine*. 2011;17(2):211-215. doi:10.1038/nm.2284

26. Chen Y, Zhu X, Zhang X, Liu B, Huang L. Nanoparticles modified with tumor-targeting scFv deliver siRNA and miRNA for cancer therapy. *Molecular Therapy*. 2010;18(9):1650-1656. doi:10.1038/mt.2010.136

27. Chen X, Peng D, Shen Y, et al. The potential combinational effect of miR-34a with celecoxib in osteosarcoma. *Anti-Cancer Drugs*. 2017;28(8). doi:10.1097/CAD.0000000000000530

28. Ma W, Xiao GG, Mao J, et al. Dysregulation of the miR-34a-SIRT1 axis inhibits breast cancer stemness. *Oncotarget*. 2015;6(12). doi:10.18632/oncotarget.3394

29. Xu X, Chen W, Miao R, et al. miR-34a induces cellular senescence via modulation of telomerase activity in human hepatocellular carcinoma by targeting FoxM1/c-Myc pathway. *Oncotarget*. 2015;6(6). doi:10.18632/oncotarget.2905

30. Cui L, Zhou H, Zhao H, et al. MicroRNA-99a induces G1-phase cell cycle arrest and suppresses tumorigenicity in renal cell carcinoma. *BMC cancer*. 2012;12(1):546. doi:10.1186/1471-2407-12-546

31. Xu C, Zeng Q, Xu W, et al. miRNA-100 Inhibits human bladder urothelial carcinogenesis by directly targeting mTOR. *Molecular Cancer Therapeutics*. 2013;12(2):207-219. doi:10.1158/1535-7163.MCT-12-0273

32. Su H, Yang J-R, Xu T, et al. MicroRNA-101, Down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. *Cancer Research*. 2009;69(3):1135. doi:10.1158/0008-5472.CAN-08-2886

33. Xu Fei, Liao Jia-Zhi, Xiang Guang-Ya, et al. MiR-101 and doxorubicin codelivered by liposomes suppressing malignant properties of hepatocellular carcinoma. *Cancer Medicine*. 2017;6(3):651-661. doi:10.1002/cam4.1016

34. Zheng F, Liao Y-J, Cai M-Y, et al. Systemic delivery of microRNA-101 potently inhibits hepatocellular carcinoma in vivo by repressing multiple targets. McKinnon P, ed. *PLoS Genetics*. 2015;11(2):1-21. doi:10.1371/journal.pgen.1004873

35. Piao L, Zhang M, Datta J, et al. Lipid-based nanoparticle delivery of pre-miR-107 inhibits the tumorigenicity of head and neck squamous cell carcinoma. *Molecular Therapy*. 2012;20(6):1261-1269. doi:10.1038/mt.2012.67

36. Huang H, Zhu Y, Li S. MicroRNA-122 mimic transfection contributes to apoptosis in HepG2 cells. *Molecular Medicine Reports*. 2015;12(5):6918-6924. doi:10.3892/mmr.2015.4254

37. Wang B, Wang H, Yang Z. MiR-122 inhibits cell proliferation and tumorigenesis of breast cancer by targeting IGF1R. Mukhopadhyay P, ed. *PLoS ONE*. 2012;7(10):e47053. doi:10.1371/journal.pone.0047053

38. Bai S, Nasser MW, Wang B, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *The Journal of Biological Chemistry*. 2009;284(46):32015-32027. doi:10.1074/jbc.M109.016774

39. Taniguchi K, Sugito N, Kumazaki M, et al. MicroRNA-124 inhibits cancer cell growth through PTB1/PKM1/PKM2 feedback cascade in colorectal cancer. *Cancer Letters*. 2015;363(1):17-27. doi:10.1016/j.canlet.2015.03.026

40. Hayward SL, Francis DM, Kholmatov P, Kidambi S. Targeted delivery of microRNA125a-5p by engineered lipid nanoparticles for the treatment of HER2 positive metastatic breast cancer. *Journal of Biomedical Nanotechnology*. 2016;12(3):554-568. doi:10.1166/jbn.2016.2194

41. Nishida N, Mimori K, Fabbri M, et al. MicroRNA-125a-5p is an independent prognostic factor in gastric cancer and inhibits the proliferation of human gastric cancer cells in combination with trastuzumab. *Clinical Cancer Research.* 2011;17(9):2725. doi:10.1158/1078-0432.CCR-10-2132

42. Tong Z, Liu N, Lin L, Guo X, Yang D, Zhang Q. miR-125a-5p inhibits cell proliferation and induces apoptosis in colon cancer via targeting BCL2, BCL2L12 and MCL1. *Biomedicine & Pharmacotherapy*. 2015;75:129-136. doi:10.1016/j.biopha.2015.07.036

43. Nishida N, Mimori K, Fabbri M, et al. MicroRNA-125a-5p is an independent prognostic factor in gastric cancer and inhibits the proliferation of human gastric

cancer cells in combination with trastuzumab. *Clinical Cancer Research.* 2011;17(9):2725. doi:10.1158/1078-0432.CCR-10-2132

44. Fu Y, Cao F. MicroRNA-125a-5p regulates cancer cell proliferation and migration through NAIF1 in prostate carcinoma. *OncoTargets and therapy*. 2015;8:3827-3835. doi:10.2147/OTT.S92314

45. Feng R, Chen X, Yu Y, et al. miR-126 functions as a tumour suppressor in human gastric cancer. *Cancer Letters*. 2010;298(1):50-63. doi:10.1016/j.canlet.2010.06.004

46. Jin M, Zhang T, Liu C, et al. miRNA-128 suppresses prostate cancer by inhibiting BMI-1 to inhibit tumor-initiating cells. *Cancer Research.* 2014;74(15):4183. doi:10.1158/0008-5472.CAN-14-0404

47. Yang D, Zhao D, Chen X. MIR-133b inhibits proliferation and invasion of gastric cancer cells by up-regulating FBN1 expression. *Cancer Biomarkers*. 2017;19(4):425-436. doi:10.3233/CBM-160421

48. Wu Y, Crawford M, Yu B, Mao Y, Nana-Sinkam SP, Lee LJ. MicroRNA delivery by cationic lipoplexes for lung cancer therapy. *Molecular pharmaceutics*. 2011;8(4):1381-1389. doi:10.1021/mp2002076

49. Wu S, Lin Y, Xu D, et al. MiR-135a functions as a selective killer of malignant glioma. *Oncogene*. 2011;31:3866. doi:10.1038/onc.2011.551

50. Silber J, Lim DA, Petritsch C, et al. miR-124 and miR-137 inhibit proliferation of glioblastoma multiforme cells and induce differentiation of brain tumor stem cells. *BMC Medicine*. 2008;6(1):14. doi:10.1186/1741-7015-6-14

51. Osaki M, Takeshita F, Sugimoto Y, et al. MicroRNA-143 regulates human osteosarcoma metastasis by regulating matrix metalloprotease-13 expression. *Molecular Therapy*. 2011;19(6):1123-1130. doi:10.1038/mt.2011.53

52. Akao Y, Nakagawa Y, Hirata I, et al. Role of anti-oncomirs miR-143 and -145 in human colorectal tumors. *Cancer Gene Therapy*. 2010;17(6):398-408.

53. He Q, Wang F, Honda T, et al. Intravenous miR-144 inhibits tumor growth in diethylnitrosamine-induced hepatocellular carcinoma in mice. *Tumour Biology.* 2017;39(10):1-8. doi:10.1177/1010428317737729

54. Ibrahim AF, Weirauch U, Thomas M, Grünweller A, Hartmann RK, Aigner A. MicroRNA replacement therapy for miR-145 and miR-33a is efficacious in a model of colon carcinoma. *Cancer Research.* 2011;71(15):5214. doi:10.1158/0008-5472.CAN-10-4645

55. Gregersen LH, Jacobsen AB, Frankel LB, Wen J, Krogh A, Lund AH. MicroRNA-145 targets YES and STAT1 in colon cancer cells. Kudla G, ed. *PLoS ONE*. 2010;5(1):e8836. doi:10.1371/journal.pone.0008836

56. Chien C-S, Chiou S-H. Cationic polyurethanes-short branch PEI-mediated delivery of Mir145 inhibited epithelial-mesenchymal transdifferentiation and cancer stem-like properties and in lung adenocarcinoma. *Cancer Research.* 2012;72(8 Supplement):3321. doi:10.1158/1538-7445.AM2012-3321

57. Zheng L, Pu J, Qi T, et al. miRNA-145 Targets v-ets erythroblastosis virus E26 oncogene homolog 1 to suppress the invasion, metastasis, and angiogenesis of gastric cancer cells. *Molecular Cancer Research.* 2013;11(2):182. doi:10.1158/1541-7786.MCR-12-0534

58. Chien C-S, Chiou S-H. Cationic polyurethanes-short branch PEI-mediated delivery of Mir145 inhibited epithelial-mesenchymal transdifferentiation and cancer stem-like properties and in lung adenocarcinoma. *Cancer Research.* 2012;72(8 Supplement):3321. doi:10.1158/1538-7445.AM2012-3321

59. Xu Q, Liu L-Z, Qian X, et al. MiR-145 directly targets p70S6K1 in cancer cells to inhibit tumor growth and angiogenesis. *Nucleic Acids Research*. 2012;40(2):761-774. doi:10.1093/nar/gkr730

60. Shi B, Sepp-Lorenzino L, Prisco M, Linsley P, deAngelis T, Baserga R. Micro RNA 145 targets the insulin receptor substrate-1 and inhibits the growth of colon cancer cells. *Journal of Biological Chemistry*. 2007;282(45):32582-32590. doi:10.1074/jbc.M702806200

61. Law PT-Y, Ching AK-K, Chan AW-H, et al. MiR-145 modulates multiple components of the insulin-like growth factor pathway in hepatocellular carcinoma. *Carcinogenesis*. 2012;33(6):1134-1141. doi:10.1093/carcin/bgs130

62. Kim S-J, Oh J-S, Shin J-Y, et al. Development of microRNA-145 for therapeutic application in breast cancer. *Journal of Controlled Release*. 2011;155(3):427-434. doi:10.1016/j.jconrel.2011.06.026

63. Spizzo R, Nicoloso MS, Lupini L, et al. miR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor-α in human breast cancer cells. *Cell Death & Differentiation*. 2009;17:246. doi:10.1038/cdd.2009.117

64. Huang S, He R, Rong M, Dang Y, Chen G. Synergistic Effect of MiR-146a Mimic and Cetuximab on Hepatocellular Carcinoma Cells. *BioMed Research International*. 2014. doi:10.1155/2014/384121

65. Katakowski M, Buller B, Zheng X, et al. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. *Cancer Letters*. 2013;335(1):201-204. doi:10.1016/j.canlet.2013.02.019

66. Li Q, Ren P, Shi P, et al. MicroRNA-148a promotes apoptosis and suppresses growth of breast cancer cells by targeting B-cell lymphoma 2. *Anti-Cancer Drugs*. 2017;28(6). doi:10.1097/CAD.0000000000000498

67. Xu Q, Jiang Y, Yin Y, et al. A regulatory circuit of miR-148a/152 and DNMT1 in modulating cell transformation and tumor angiogenesis through IGF-IR and IRS1. *Journal of Molecular Cell Biology*. 2013;5(1):3-13. doi:10.1093/jmcb/mjs049

68. Arora S, Swaminathan SK, Kirtane A, et al. Synthesis, characterization, and evaluation of poly (D,L-lactide-co-glycolide)-based nanoformulation of miRNA-150: potential implications for pancreatic cancer therapy. *International Journal of Nanomedicine*. 2014;9:2933-2942. doi:10.2147/IJN.S61949

69. Kouri FM, Hurley LA, Daniel WL, et al. miR-182 integrates apoptosis, growth, and differentiation programs in glioblastoma. *Genes & Development*. 2015;29(7):732-745. doi:10.1101/gad.257394.114

70. Zhou L, Liu S, Han M, et al. MicroRNA-185 induces potent autophagy via AKT signaling in hepatocellular carcinoma. *Tumour Biology*. 2017;39(2):1010428317694313. doi:10.1177/1010428317694313

71. Xu J, Ai Q, Cao H, Liu Q. MiR-185-3p and miR-324-3p Predict radiosensitivity of nasopharyngeal carcinoma and modulate cancer cell growth and apoptosis by targeting SMAD7. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*. 2015;21:2828-2836. doi:10.12659/MSM.895660

72. Fan Q, Hu X, Zhang H, et al. MiR-193a-3p is an important tumour suppressor in lung cancer and directly targets KRAS. *Cellular Physiology and Biochemistry*. 2017;44(4):1311-1324. doi:10.1159/000485491

73. Wang Z-S, Zhong M, Bian Y-H, et al. MicroRNA-187 inhibits tumor growth and invasion by directly targeting CD276 in colorectal cancer. *Oncotarget*. 2016;7(28):44266-44276. doi:10.18632/oncotarget.10023

74. Liu Y, Wu X, Gao Y, et al. Aptamer-functionalized peptide H(3)CR(5)C as a novel nanovehicle for codelivery of fasudil and miRNA-195 targeting hepatocellular carcinoma. *International Journal of Nanomedicine*. 2016;11:3891-3905. doi:10.2147/IJN.S108128

75. Hurteau GJ, Carlson JA, Spivack SD, Brock GJ. Overexpression of the microRNA hsa-miR-200c leads to reduced expression of transcription factor 8 and increased expression of E-cadherin. *Cancer Research* 2007;67(17):7972. doi:10.1158/0008-5472.CAN-07-1058

76. Xie Y, Wehrkamp CJ, Li J, et al. Delivery of miR-200c mimic with poly(amido amine) CXCR4 antagonists for combined inhibition of cholangiocarcinoma cell invasiveness. *Molecular pharmaceutics*. 2016;13(3):1073-1080. doi:10.1021/acs.molpharmaceut.5b00894

77. Cittelly DM, Dimitrova I, Howe EN, et al. Restoration of miR-200c to ovarian cancer reduces tumor burden and increases sensitivity to paclitaxel. *Molecular cancer therapeutics*. 2012;11(12):2556-2565. doi:10.1158/1535-7163.MCT-12-0463

78. Peng F, Jiang J, Yu Y, et al. Direct targeting of SUZ12/ROCK2 by miR-200b/c inhibits cholangiocarcinoma tumourigenesis and metastasis. *British Journal of Cancer*. 2013;109(12):3092-3104. doi:10.1038/bjc.2013.655

79. Ding X, Park SI, McCauley LK, Wang C-Y. Signaling between transforming growth factor β (TGF-β) and transcription factor SNAI2 represses expression of microRNA miR-203 to promote epithelial-mesenchymal transition and tumor metastasis. *Journal of Biological Chemistry*. 2013;288(15):10241-10253. doi:10.1074/jbc.M112.443655

80. Bo Juanjie, Yang Guoliang, Huo Kailing, et al. microRNA-203 suppresses bladder cancer development by repressing bcl-w expression. *The FEBS Journal*. 2011;278(5):786-792. doi:10.1111/j.1742-4658.2010.07997.x

81. Wu Heng, Tao Junyan, Li Xiaolei, et al. MicroRNA-206 prevents the pathogenesis of hepatocellular carcinoma by modulating expression of met proto-oncogene and cyclin-dependent kinase 6 in mice. *Hepatology*. 2017;66(6):1952-1967. doi:10.1002/hep.29374

82. Yan G-J, Yu F, Wang B, et al. MicroRNA miR-302 inhibits the tumorigenicity of endometrial cancer cells by suppression of Cyclin D1 and CDK1. *Cancer Letters*. 2014;345(1):39-47. doi:10.1016/j.canlet.2013.11.023

83. Lv Q, Hu J-X, Li Y-J, et al. MiR-320a effectively suppresses lung adenocarcinoma cell proliferation and metastasis by regulating STAT3 signals. *Cancer Biology & Therapy*. 2017;18(3):142-151. doi:10.1080/15384047.2017.1281497

84. Liang Z, Wu H, Xia J, et al. Involvement of miR-326 in chemotherapy resistance of breast cancer through modulating expression of multidrug resistance-associated protein 1. *Biochemical pharmacology*. 2010;79(6):817-824. doi:10.1016/j.bcp.2009.10.017

85. Yuan J, Ji H, Xiao F, et al. MicroRNA-340 inhibits the proliferation and invasion of hepatocellular carcinoma cells by targeting JAK1. *Biochemical and Biophysical Research Communications*. 2017;483(1):578-584. doi:10.1016/j.bbrc.2016.12.102

86. Yeh L-Y, Liu C-J, Wong Y-K, Chang C, Lin S-C, Chang K-W. miR-372 inhibits p62 in head and neck squamous cell carcinoma in vitro and in vivo. *Oncotarget*. 2015;6(8):6062-6075. doi:10.18632/oncotarget.3340

87. Xue H-Y, Liu Y, Liao J-Z, et al. Gold nanoparticles delivered miR-375 for treatment of hepatocellular carcinoma. *Oncotarget*. 2016;7(52):86675-86686. doi:10.18632/oncotarget.13431

88. Isozaki Y, Hoshino I, Akutsu Y, et al. Usefulness of microRNA-375 as a prognostic and therapeutic tool in esophageal squamous cell carcinoma. *International Journal of Oncology*. 2015;46(3):1059+. doi:10.3892/ijo.2014.2789

89. Seki N. Tumor suppressive microRNA-375 regulates lactate dehydrogenase B in maxillary sinus squamous cell carcinoma. *International Journal of Oncology*. September 2011. doi:10.3892/ijo.2011.1196

90. Fei B, Wu H. MiR-378 inhibits progression of human gastric cancer MGC-803 cells by targeting MAPK1 in vitro. *Oncology Research Featuring Preclinical and Clinical Cancer Therapeutics*. 2013;20(12):557-564. doi:doi:10.3727/096504013X13775486749254

91. Chen T, Ren H, Thakur A, et al. miR-382 inhibits tumor progression by targeting SETD8 in non-small cell lung cancer. *Biomedicine & Pharmacotherapy*. 2017;86:248-253. doi:10.1016/j.biopha.2016.12.007

92. Hershkovitz-Rokah O, Modai S, Pasmanik-Chor M, et al. Restoration of miR-424 suppresses BCR–ABL activity and sensitizes CML cells to imatinib treatment. *Cancer Letters*. 2015;360(2):245-256. doi:10.1016/j.canlet.2015.02.031

93. Noonan EJ, Place RF, Basak S, Pookot D, Li L-C. miR-449a causes Rb-dependent cell cycle arrest and senescence in prostate cancer cells. *Oncotarget*. 2010;1(5):349-358.

94. Zhu H, Wu H, Liu X, et al. Role of MicroRNA miR-27a and miR-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. *Biochemical pharmacology*. 2008;76(5):582-588. doi:10.1016/j.bcp.2008.06.007

95. Nan Y, Han L, Zhang A, et al. MiRNA-451 plays a role as tumor suppressor in human glioma cells. *Brain Research*. 2010;1359:14-21. doi:10.1016/j.brainResearch2010.08.074

96. Peng Y, Dai Y, Hitchcock C, et al. Insulin growth factor signaling is regulated by microRNA-486, an underexpressed microRNA in lung cancer. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(37):15043-15048. doi:10.1073/pnas.1307107110

97. Denoyelle C, Lambert B, Meryet-Figuière M, et al. miR-491-5p-induced apoptosis in ovarian carcinoma depends on the direct inhibition of both BCL-XL and EGFR leading to BIM activation. *Cell Death & Disease*. 2014;5:e1445.

98. Yan L, Yao J, Qiu J. miRNA-495 suppresses proliferation and migration of colorectal cancer cells by targeting FAM83D. *Biomedicine & Pharmacotherapy*. 2017;96:974-981. doi:10.1016/j.biopha.2017.11.138

99. Bao G, Wang N, Li R, Xu G, Liu P, He B. MiR-508-5p inhibits the progression of glioma by targeting glycoprotein non-metastatic melanoma B. *Neurochemical Research*. 2016;41(7):1684-1690. doi:10.1007/s11064-016-1884-2

100. Wang F, Xue X, Wei J, et al. hsa-miR-520h downregulates ABCG2 in pancreatic cancer cells to inhibit migration, invasion, and side populations. *British Journal of Cancer*. 2010;103:567.

101. Wang S, Zhang J, Wang Y, Chen M. Hyaluronic acid-coated PEI-PLGA nanoparticles mediated co-delivery of doxorubicin and miR-542-3p for triple negative breast cancer therapy. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2016;12(2):411-420. doi:10.1016/j.nano.2015.09.014

102. Bayraktar R, Pichler M, Kanlikilicer P, et al. MicroRNA 603 acts as a tumor suppressor and inhibits triple-negative breast cancer tumorigenesis by targeting elongation factor 2 kinase. *Oncotarget*. 2017;8(7):11641-11658. doi:10.18632/oncotarget.14264

103. Xiao F, Qiu H, Cui H, et al. MicroRNA-885-3p inhibits the growth of HT-29 colon cancer cell xenografts by disrupting angiogenesis via targeting BMPR1A

and blocking BMP/Smad/Id1 signaling. *Oncogene*. 2014;34:1968. doi:10.1038/onc.2014.134

104. Zhang L, Sullivan PS, Goodman JC, Gunaratne PH, Marchetti D. MicroRNA-1258 suppresses breast cancer brain metastasis by targeting heparanase. *Cancer Research* 2011;71(3):645. doi:10.1158/0008-5472.CAN-10-1910

REFERENCES

Alvarez-Saavedra, E., & Horvitz, H. R. (2010). Many families of C. elegans microRNAs are not essential for development or viability. *Current Biology*, *20*(4), 367–373. https://doi.org/10.1016/j.cub.2009.12.051

Bader, A. G., Brown, D., Stoudemire, J., & Lammers, P. (2011). Developing therapeutic microRNAs for cancer. *Gene Therapy*, *18*(12), 1121–1126. https://doi.org/10.1038/gt.2011.79

Bazzini, A. A., Lee, M. T., & Giraldez, A. J. (2012). Ribosome profiling shows that miR-430 reduces translation before causing mRNA decay in zebrafish. *Science (New York, N.Y.)*, *336*(6078), 233–237. https://doi.org/10.1126/science.1215704

Beg, M. S., Brenner, A. J., Sachdev, J., Borad, M., Kang, Y.-K., Stoudemire, J., Smith, S., Bader, A.G., Kim, S., & Hong, D. S. (2017). Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Investigational New Drugs*, *35*(2), 180–188. https://doi.org/10.1007/s10637-016-0407-y

Behm-Ansmant, I. Rehwinkel, J., Doerks, T., Stark, A., Bork, P., & Izaurralde, E. (2006). mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes & Development*, *20*(14), 1885–1898. https://doi.org/10.1101/gad.1424106

Borchert, G. M., Lanier, W., & Davidson, B. L. (2006). RNA polymerase III transcribes human microRNAs. *Nature Structural & Molecular Biology*, *13*, 1097. http://doi.org/10.1038/nsmb1167

Brennecke, J., Hipfner, D. R., Stark, A., Russell, R. B., & Cohen, S. M. (2003). bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell*, *113*(1), 25–36. https://doi.org/10.1016/S0092-8674(03)00231-9

Calin, G. A., Dumitru, C. D., Shimizu, M., Bichi, R., Zupo, S., Noch, E., Alder, H., Rattan, S., Keating, M., Rai, K., Rassenti, L., Kipps, T., Negrini, M., Bullrich, F., & Croce, C. M. (2002). Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proceedings of the National Academy of Sciences*, *99*(24), 15524. https://doi.org/10.1073/pnas.242606799

Calin, G. A., Sevignani, C., Dumitru, C. D., Hyslop, T., Noch, E., Yendamuri, S., Masayoshi, S., Rattan, S., Bullrich, F., Negrini, M., & Croce, C. M. (2004). Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(9), 2999. https://doi.org/10.1073/pnas.0307323101

Cha, S.-T., Chen, P.-S., Johansson, G., Chu, C.-Y., Wang, M.-Y., Jeng, Y.-M., Yu, S.-L., Chen, J.-S., Chang, K.-J., Jee, S.-H., Tan, C.-T., Lin. M.-T., & Kuo, M.-L. (2010). MicroRNA-519c suppresses hypoxia-inducible factor-1α expression and tumor angiogenesis. *Cancer Research*, *70*(7), 2675. https://doi.org/10.1158/0008-5472.CAN-09-2448

Chan, H. S. L. (2007). *Understanding cancer therapies* (1st ed.). Jackson: University Press of Mississippi.

Chang, C.-J., Chao, C.-H., Xia, W., Yang, J.-Y., Xiong, Y., Li, C.-W., Yu, W.-H., Rehman, S. K., Hsu, J. L., Lee, H.-H., Liu, M., Chen, C.-T., Yu, D., & Hung, M.-C. (2011). p53 regulates epithelial–mesenchymal transition and stem cell properties through modulating miRNAs. *Nature Cell Biology*, *13*, 317. https://doi.org/10.1038/ncb2173

Chang, T.-C., Wentzel, E. A., Kent, O. A., Ramachandran, K., Mullendore, M., Lee, K. H., Feldmann, G., Yamakuchi, M., Ferlito, M., Lowenstein, C. J., Arking, D. E., Beer, M. A., Marita, A., & Mendell, J. T. (2007). Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. *Molecular Cell*, *26*(5), 745–752. https://doi.org/10.1016/j.molcel.2007.05.010

Chang, T.-C., Yu, D., Lee, Y.-S., Wentzel, E. A., Arking, D. E., West, K. M., Dang, C. V., Thomas-Tickhonenko, A., & Mendell, J. T. (2007). Widespread microRNA repression by Myc contributes to tumorigenesis. *Nature Genetics*, *40*, 43. https://doi.org/10.1038/ng.2007.30

Cimmino, A., Calin, G. A., Fabbri, M., Iorio, M. V., Ferracin, M., Shimizu, M., Wojcik, S. E., Aqeilan, R. I., Zupo, S., Dono, M., Rassenti, L., Alder, H., Volinia, S., Liu, C.-G., Kipps, T. J., Negrini, M., & Croce, C. M. (2005). miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(39), 13944. https://doi.org/10.1073/pnas.0506654102

Cohen, A., Burgos-Aceves, M. A., & Smith, Y. (2018). MicroRNAs downregulation in cancer is associated with guanine enrichment in the terminal loop sequences

of their precursors. *Bentham Science*, *7*(1), 20–27. https://doi.org/10.2174/2211536607666171213122032

Daige, C. L., Wiggins, J. F., Priddy, L., Nelligan-Davis, T., Zhao, J., & Brown, D. (2014). Systemic delivery of a miR34a mimic as a potential therapeutic for liver cancer. *Molecular Cancer Therapeutics*, *13*(10), 2352. https://doi.org/10.1158/1535-7163.MCT-14-0209

Dang, C. V., Resar, L. M. S., Emison, E., Kim, S., Li, Q., Prescott, J. E., Wonsey, D., & Zeller, K. (1999). Function of the c-Myc oncogenic transcription factor. *Experimental Cell Research*, *253*(1), 63–77. https://doi.org/10.1006/excr.1999.4686

Ding, X., Park, S. I., McCauley, L. K., & Wang, C.-Y. (2013). Signaling between transforming growth factor β (TGF-β) and transcription factor SNAI2 represses expression of microRNA miR-203 to promote epithelial-mesenchymal transition and tumor metastasis. *Journal of Biological Chemistry*, *288*(15), 10241–10253. https://doi.org/10.1074/jbc.M112.443655

Donzelli, S., Mori, F., Bellissimo, T., Sacconi, A., Casini, B., Frixa, T., Roscilli, G., Aurisicchio, L., Facciolo, F., Pompili, A., Facciolo, F., Pompili, A., Carosi, M. A., Pescarmona, E., Segatto, O., Pond, G., Muti, P., Terela, S., Strano, S., Yarden, Y., & Blandino, G. (2015). Epigenetic silencing of miR-145-5p contributes to brain metastasis. *Oncotarget*, *6*(34), 35183–35201. https://doi.org/10.18632/oncotarget.5930

Eulalio, A., Rehwinkel, J., Stricker, M., Huntzinger, E., Yang, S.-F., Doerks, T., Doner, S., Bork, P., Boutros M., & Izaurralde, E. (2007). Target-specific requirements for enhancers of decapping in miRNA-mediated gene silencing. *Genes & Development*, *21*(20), 2558–2570. https://doi.org/10.1101/gad.443107

Fabian, M. R., Sundermeier, T. R., & Sonenberg, N. (2010). Understanding how miRNAs post-transcriptionally regulate gene expression. In R. E. Rhoads (Ed.), *miRNA regulation of the translational machinery* (pp. 1–20). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-03103-8_1

Fazi, F., Racanicchi, S., Zardo, G., Starnes, L. M., Mancini, M., Travaglini, L., Diverio, D., Ammatuna, E., Cimino, G., Lo-Coco, F., Grignani, F., & Nervi, C. (2007). Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. *Cancer Cell*, *12*(5), 457–466. https://doi.org/10.1016/j.ccr.2007.09.020

Feinberg, A. P., & Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature*, *301*, 89. https://doi.org//10.1038/301089a0

Ferguson, R. D., Alikhani, N., Vijaykumar, A., Firez, Y., Cannata, D., & Yakar, S. (2012). IGF-1 cellular action and its relationship to cancer: Evidence from in vitro and in vivo studies. In D. LeRoith (Ed.), *Insulin-like growth factors and cancer: From basic biology to therapeutics* (pp. 105–146). Boston, MA: Springer. https://doi.org/10.1007/978-1-4614-0598-6

Ferrara, N. (2008). Pathophysiology and clinical implications of vascular endothelial growth factor. In D. Marmé & N. Fusenig (Eds.), *Tumor angiogenesis: Basic mechanisms and cancer therapy* (pp. 89–108). Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-540-33177-3_5

Gillies, J. K., & Lorimer, I. A. J. (2007). Regulation of p27Kip1 by miRNA 221/222 in Glioblastoma. *Cell Cycle*, *6*(16), 2005–2009. https://doi.org/10.4161/cc.6.16.4526

Giraldez, A. J., Mishima, Y., Rihel, J., Grocock, R. J., Van Dongen, S., Inoue, K., Enright, A. J., & Schier, A. F. (2006). Zebrafish miR-430 promotes deadenylation and clearance of maternal mRNAs. *Science*, *312*(5770), 75. https://doi.org/10.1126/science.1122689

Gregory, P. A., Bert, A. G., Paterson, E. L., Barry, S. C., Tsykin, A., Farshid, G., Vadas, M. A., Khew-Goodall, Y., & Goodall, G. J. (2008). The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nature Cell Biology*, *10*, 593. https://doi.org/10.1038/ncb1722

Gregory, R. I., Yan, K., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., & Shiekhattar, R. (2004). The Microprocessor complex mediates the genesis of microRNAs. *Nature*, *432*, 235. https://doi.org/10.1038/nature03120

Han, L., Witmer, P. D. W., Casey, E., Valle, D., & Sukumar, S. (2007). DNA methylation regulates microRNA expression. *Cancer Biology & Therapy*, *6*(8), 1290–1294. https://doi.org/10.4161/cbt.6.8.4486

Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, *100*(1), 57–70. https://doi.org/10.1016/S0092-8674(00)81683-9

Herman, J. G., Latif, F., Weng, Y., Lerman, M. I., Zbar, B., Liu, S., Samid, D., Duan, D. S., Gnarra, J. R., & Linehan, W. M. (1994). Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proceedings of the National Academy of Sciences*, *91*(21), 9700. https://doi.org/10.1073/pnas.91.21.9700

Hosseinahli, N., Aghapour, M., Duijf, P. H. G., & Baradaran, B. (2018). Treating cancer with microRNA replacement therapy: A literature review. *Journal of Cellular Physiology*, *233*(8), 5574–5588. https://doi.org/10.1002/jcp.26514

Hutvágner, G., & Zamore, P. D. (2002). A microRNA in a multiple-turnover RNAi enzyme complex. *Science*, *297*(5589), 2056. https://doi.org/10.1126/science.1073827

Iliou, M. S., da Silva-Diz, V., Carmona, F. J., Ramalho-Carvalho, J., Heyn, H., Villanueva, A., Muñoz, P., & Esteller, M. (2013). Impaired DICER1 function promotes stemness and metastasis in colon cancer. *Oncogene*, *33*, 4003. https://doi.org//10.1038/onc.2013.398

Ivkovic, T. C., Voss, G., Cornella, H., & Ceder, Y. (2017). MicroRNAs as cancer therapeutics: A step closer to clinical application. *Cancer Letters*, *407*, 113–122. https://doi.org/10.1016/j.canlet.2017.04.007

Karube, Y., Tanaka, H., Osada, H., Tomida, S., Tatematsu, Y., Yanagisawa, K., Yatabe, Y., Takamizawa, J., Miyoshi, S., Mitsudomi, T., Takahashi, T. (2005). Reduced expression of Dicer associated with poor prognosis in lung cancer patients. *Cancer Science*, *96*(2), 111–115. https://doi.org/10.1111/j.1349-7006.2005.00015.x

Kelnar, K., Peltier, H. J., Leatherbury, N., Stoudemire, J., & Bader, A. G. (2014). Quantification of therapeutic miRNA mimics in whole blood from nonhuman primates. *Analytical Chemistry*, *86*(3), 1534–1542. https://doi.org/10.1021/ac403044t

Kim, T., Veronese, A., Pichiorri, F., Lee, T. J., Jeon, Y.-J., Volinia, S., Pineau, P., Marchio, A., Palatini, J., Suh, S.-S., Alder, H., Liu, C.-G., Dejean, A., & Croce, C. M. (2011). p53 regulates epithelial–mesenchymal transition through microRNAs targeting ZEB1 and ZEB2. *The Journal of Experimental Medicine*, *208*(5), 875. https://doi.org/10.1084/jem.20110235

Kozomara, A., & Griffiths-Jones, S. (2011). miRBase: Integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Research*, *39*(Database issue), D152–D157. https://doi.org/10.1093/nar/gkq1027

Kumar, M. S., Lu, J., Mercer, K. L., Golub, T. R., & Jacks, T. (2007). Impaired microRNA processing enhances cellular transformation and tumorigenesis. *Nature Genetics*, *39*, 673. https://doi.org/10.1038/ng2003

Lai, E. C., Tam, B., & Rubin, G. M. (2005). Pervasive regulation of Drosophila Notch target genes by GY-box-, Brd-box-, and K-box-class microRNAs. *Genes & Development*, *19*(9), 1067–1080. https://doi.org/10.1101/gad.1291905

Lee, R. C., Feinbaum, R. L., & Ambros, V. (1993). The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell*, *75*(5), 843–854. https://doi.org/10.1016/0092-8674(93)90529-Y

Lee, Y., Kim, M., Han, J., Yeom, K.-H., Lee, S., Baek, S. H., & Kim, V. N. (2004). MicroRNA genes are transcribed by RNA polymerase II. *The EMBO Journal*, *23*(20), 4051–4060. https://doi.org/10.1038/sj.emboj.7600385

Lehmann, U., Hasemeier, B., Christgen, M., Müller, M., Römermann, D., Länger, F., & Kreipe, H. (2007). Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. *The Journal of Pathology*, *214*(1), 17–24. https://doi.org/10.1002/path.2251

Lei, Z., Li, B., Yang, Z., Fang, H., Zhang, G.-M., Feng, Z.-H., & Huang, B. (2009). Regulation of HIF-1α and VEGF by miR-20b tunes tumor cells to adapt to the alteration of oxygen concentration. *PLoS ONE*, *4*(10), e7629. https://doi.org/10.1371/journal.pone.0007629

Liu, J., Carmell, M. A., Rivas, F. V., Marsden, C. G., Thomson, J. M., Song, J.-J., Hammond, S. M., Joshua-Tor, L., & Hannon, G. J. (2004). Argonaute2 is the catalytic engine of mammalian RNAi. *Science*, *305*(5689), 1437. https://doi.org/10.1126/science.1102513

Liu, S., Shen, T., Huynh, L., Klisovic, M. I., Rush, L. J., Ford, J. L., Yu, L., Chunhui, L., Vukosavljevic, T., Whitman, S. P., Chang, K.-S., Byrd, J. C., Perrotti, D., Plass, C., & Marcucci, G. (2005). Interplay of RUNX1/MTG8 and DNA methyltransferase 1 in acute myeloid leukemia. *Cancer Research*, *65*(4), 1277. https://doi.org/10.1158/0008-5472.CAN-04-4532

Liu, X., Fang, H., Chen, H., Jiang, X., Fang, D., Wang, Y., & Zhu, D. (2012). An artificial miRNA against HPSE suppresses melanoma invasion properties, correlating with a down-regulation of chemokines and MAPK phosphorylation. *PLoS ONE*, *7*(6), e38659. https://doi.org/10.1371/journal.pone.0038659

Lu, J., Getz, G., Miska, E. A., Alvarez-Saavedra, E., Lamb, J., Peck, D., Sweet-Cordero, A., Ebert, B. L., Mak, R. H., Ferrando, A. A., Downing, J. R., Jacks, T., Horvitz, H. R., Golub, T. R. (2005). MicroRNA expression profiles classify human cancers. *Nature*, *435*, 834. https://doi.org/10.1038/nature03702

Lujambio, A., Calin, G. A., Villanueva, A., Ropero, S., Sánchez-Céspedes, M., Blanco, D., Montuenga, L. M., Rossi, S., Nicoloso, M. S., Faller, W. J., Gallagher, W. M., Eccles, S. A., Croce, C. M., & Esteller, M. (2008). A microRNA DNA methylation signature for human cancer metastasis. *Proceedings of the National Academy of Sciences*, *105*(36), 13556. https://doi.org/10.1073/pnas.0803055105

Lund, E., Güttinger, S., Calado, A., Dahlberg, J. E., & Kutay, U. (2004). Nuclear export of microRNA precursors. *Science*, *303*(5654), 95. https://doi.org/10.1126/science.1090599

MacDiarmid, J. A., Mugridge, N. B., Weiss, J. C., Phillips, L., Burn, A. L., Paulin, R. P., Haasdyk, J. E., Dickson, K.-A., Brahmbhatt, V. N., Pattison, S. T., James, A. C., Al Bakri, G., Straw, R. C., Stillman, B., Graham, R. M., & Brahmbhatt, H. (2007). Bacterially derived 400 nm particles for encapsulation and cancer cell targeting of chemotherapeutics. *Cancer Cell*, *11*(5), 431–445. https://doi.org/10.1016/j.ccr.2007.03.012

Mathonnet, G., Fabian, M. R., Svitkin, Y. V., Parsyan, A., Huck, L., Murata, T., Biffo, S., Merrick, W. C., Darzynkiewicz, E., Pillai, R. S., Filipowicz, W., Duchaine, T. F., & Sonenberg, N. (2007). MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. *Science*, *317*(5845), 1764. https://doi.org/10.1126/science.1146067

Meister, G., Landthaler, M., Peters, L., Chen, P. Y., Urlaub, H., Lührmann, R., & Tuschl, T. (2005). Identification of novel Argonaute-associated proteins. *Current Biology*, *15*(23), 2149–2155. https://doi.org/10.1016/j.cub.2005.10.048

Melo, S. A., Moutinho, C., Ropero, S., Calin, G. A., Rossi, S., Spizzo, R., Fernandez, A. F., Davalos, V., Vilanueva, A., Montoya, G., Yamamoto, H., Schwartz, S., & Esteller, M. (2010). A genetic defect in exportin-5 traps precursor microRNAs in the nucleus of cancer cells. *Cancer Cell*, *18*(4), 303–315. https://doi.org/10.1016/j.ccr.2010.09.007

Meng, X., Wu, J., Pan, C., Wang, H., Ying, X., Zhou, Y., Yu, H., Zuo, Y., Pan, Z., Liu, R.-Y., & Huang, W. (2013). Genetic and epigenetic down-regulation of microRNA-212 promotes colorectal tumor metastasis via dysregulation of MnSOD. *Gastroenterology*, *145*(2), 426-436.e6. https://doi.org/10.1053/j.gastro.2013.04.004

Merritt, W. M., Lin, Y. G., Han, L. Y., Kamat, A. A., Spannuth, W. A., Schmandt, R., Urbauer, D., Pennacchio, L. A., Cheng, J.-F., Nick, A. M., Deavers, M. T., Mourad-Zeidan, A., Wang, H., Mueller, P., Lenburg, M. E., Gray, J. W., Mok, S., Birrer, M. J., Lopez-Berestein, G., Coleman, R. L., Bar-Eli, M., & Sood, A. K. (2008). Dicer, Drosha, and outcomes in patients with ovarian cancer. *New England Journal of Medicine*, *359*(25), 2641–2650. https://doi.org/10.1056/NEJMoa0803785

Mraz, M., Pospisilova, S., Malinova, K., Slapak, I., & Mayer, J. (2009). MicroRNAs in chronic lymphocytic leukemia pathogenesis and disease subtypes. *Leukemia & Lymphoma*, *50*(3), 506–509. https://doi.org/10.1080/10428190902763517

Nayer, H., Mahyar, A., Duijf, P. H. G., & Behzad, B. (2018). Treating cancer with microRNA replacement therapy: A literature review. *Journal of Cellular Physiology*, *233*(8), 5574–5588. https://doi.org/10.1002/jcp.26514

Ng, H.-H., Zhang, Y., Hendrich, B., Johnson, C. A., Turner, B. M., Erdjument-Bromage, H., Tempst, P., Reinberg, D., & Bird, A. (1999). MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nature Genetics*, *23*, 58. https://doi.org//10.1038/12659

Nicholson, R., Gee, J. M., & Harper, M. (2001). EGFR and cancer prognosis. *European Journal of Cancer*, *37*, 9–15. https://doi.org/10.1016/S0959-8049(01)00231-3

Nie, J., Liu, L., Zheng, W., Chen, L., Wu, X., Xu, Y., Du, X., & Han, W. (2012). MicroRNA-365, down-regulated in colon cancer, inhibits cell cycle progression and promotes apoptosis of colon cancer cells by probably targeting cyclin D1 and Bcl-2. *Carcinogenesis*, *33*(1), 220–225. https://doi.org/10.1093/carcin/bgr245

O'Donnell, K. A., Wentzel, E. A., Zeller, K. I., Dang, C. V., & Mendell, J. T. (2005). c-Myc-regulated microRNAs modulate E2F1 expression. *Nature*, *435*, 839. https://doi.org/10.1038/nature03677

Olivier, M., Petitjean, A., Fromentel, C. C. de, & Hainaut, P. (2010). TP53 mutations in human cancers: Selection versus mutagenesis. In A. Ayed & T. Hupp (Eds.), *p53* (pp. 1–18). Boston: Springer. Retrieved from https://doi-org/10.1007/978-1-4419-8231-5

Peng, Y., Dai, Y., Hitchcock, C., Yang, X., Kassis, E. S., Liu, L., Luo, Z., Sun, H.-L., Cui, R., Wei, H., Kim, T., Lee, T. J., Jeon, Y.-J., Nuovo, G. J., Violina, S., He, Q., Yu, J., Nana-

Sinkam, P., & Croce, C. M. (2013). Insulin growth factor signaling is regulated by microRNA-486, an underexpressed microRNA in lung cancer. *Proceedings of the National Academy of Sciences*, *110*(37), 15043. https://doi.org/10.1073/pnas.1307107110

Petersen, C. P., Bordeleau, M.-E., Pelletier, J., & Sharp, P. A. (2006). Short RNAs repress translation after initiation in mammalian cells. *Molecular Cell*, *21*(4), 533–542. https://doi.org/10.1016/j.molcel.2006.01.031

Pichiorri, F., Suh, S.-S., Rocci, A., De Luca, L., Taccioli, C., Santhanam, R., Zhou, W., Benson, D. M., Hofmainster, C., Alder, H., Garofalo, M., Di Leva, G., Violinia, S., Lin, H.-J., Perrotti, D., Kuehl, M., Aqeilan, R. I., Palumbo, A., & Croce, C. M. (2010). Downregulation of p53-inducible microRNAs 192, 194, and 215 impairs the p53/MDM2 autoregulatory loop in multiple myeloma development. *Cancer Cell*, *18*(4), 367–381. https://doi.org/10.1016/j.ccr.2010.09.005

Redondo, M., Fùnez, R., & Esteban, F. (2009). Apoptosis in the development and treatment of laryngeal cancer: Role of p53, Bcl-2 and clusterin. In G.G. Chen & P.B.S. Lai (Eds.), *Apoptosis in carcinogenesis and chemotherapy: Apoptosis in cancer* (pp. 237–249). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-9597-9_10

Reid, G., Pel, M. E., Kirschner, M. B., Cheng, Y. Y., Mugridge, N., Weiss, J., Williams, M., Wright, C., Edelman, J. J.B., Vallely, M. P., McCaughan, B. C., Klebe, S., Brahmbhatt, H., MacDiarmid, J. A., & van Zandwijk, N. (2013). Restoring expression of miR-16: A novel approach to therapy for malignant pleural mesothelioma. *Annals of Oncology*, *24*(12), 3128–3135. https://doi.org/10.1093/annonc/mdt412

Roche, J. (2018). The epithelial-to-mesenchymal transition in cancer. In J. Roche (Ed.), *The epithelial-to-mesenchymal transition (EMT) in cancer* (1st ed., pp. 1–4). Basel, Switzerland: MDPI. Retrieved from https://doi.org/10.3390/cancers10020052

Rupaimoole, R., & Slack, F. J. (2017). MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nature Reviews Drug Discovery*, *16*, 203. https://doi.org/10.1038/nrd.2016.246

Sacconi, A., Biagioni, F., Canu, V., Mori, F., Di Benedetto, A., Lorenzon, L., Ercolani, C., Di Agostino, S., Cambria, A. M., Germoni, S., Grasso, G., Blandino, R., Panebianco, V., Ziparo, V., Federici, O., Muti, P., Strano, S., Carboni, F., Mottolese, M., Diodoro, M., Pescarmona, E., Garofalo, A., & Blandino, G.

(2012). miR-204 targets Bcl-2 expression and enhances responsiveness of gastric cancer. *Cell Death & Disease*, *3*, e423. https://doi.org/10.1038/cddis.2012.160

Saito, Y., Liang, G., Egger, G., Friedman, J. M., Chuang, J. C., Coetzee, G. A., & Jones, P. A. (2006). Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell*, *9*(6), 435–443. https://doi.org/10.1016/j.ccr.2006.04.020

Schultz, R. M. (2005). Dawn of a new era in molecular cancer therapeutics. In R. M. Schultz (Ed.), *Advances in targeted cancer therapy* (Vol. 63, pp. 8–19). Basel, Switzerland: Birkhäuser Verlag.

Sun, J., Wei, Q., Zhou, Y., Wang, J., Liu, Q., & Xu, H. (2017). A systematic analysis of FDA-approved anticancer drugs. *BMC Systems Biology*, *11*(Suppl 5), 87. https://doi.org/10.1186/s12918-017-0464-7

The Cancer Genome Atlas Research Network. (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*, *455*, 1061.

Thomson, J. M., Newman, M., Parker, J. S., Morin-Kensicki, E. M., Wright, T., & Hammond, S. M. (2006). Extensive post-transcriptional regulation of microRNAs and its implications for cancer. *Genes & Development*, *20*(16), 2202–2207. https://doi.org/10.1101/gad.1444406

Tolcher, A. W., Rodrigueza, W. V., Rasco, D. W., Patnaik, A., Papadopoulos, K. P., Amaya, A., Moore, T. D., Gaylor, S. K., Bisgaier, C. L., Sooch, M. P., Woolliscroft, M. J., & Messmann, R. A. (2014). A phase 1 study of the BCL2-targeted deoxyribonucleic acid inhibitor (DNAi) PNT2258 in patients with advanced solid tumors. *Cancer Chemotherapy and Pharmacology*, *73*(2), 363–371. https://doi.org/10.1007/s00280-013-2361-0

Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J., & Jemal, A. (2015). Global cancer statistics, 2012. *CA: A Cancer Journal for Clinicians*, *65*(2), 87–108. https://doi.org/10.3322/caac.21262

Trang, P., Wiggins, J. F., Daige, C. L., Cho, C., Omotola, M., Brown, D., Weidhaas, J. B., Bader, A. G., Slack, F. J. (2011). Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. *Molecular Therapy*, *19*(6), 1116–1122. https://doi.org/10.1038/mt.2011.48

Tsang, J. S., Ebert, M. S., & van Oudenaarden, A. (2010). Genome-wide dissection of microRNA functions and cotargeting networks using gene set signatuResearch *Molecular Cell*, *38*(1), 140–153. https://doi.org/10.1016/j.molcel.2010.03.007

Turchinovich, A., Weiz, L., Langheinz, A., & Burwinkel, B. (2011). Characterization of extracellular circulating microRNA. *Nucleic Acids Research*, *39*(16), 7223–7233. https://doi.org/10.1093/nar/gkr254

van Zandwijk, N., Pavlakis, N., Kao, S. C., Linton, A., Boyer, M. J., Clarke, S., Huynh, Y., Chrzanowska, A., Fulham, M. J., Bailey, D. L., Cooper, W. A., Kritharides, L., Ridley, L., Pattison, S. T., MacDiarmid, J., Brahmbhatt, H., & Reid, G. (2017). Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: A first-in-man, phase 1, open-label, dose-escalation study. *The Lancet Oncology*, *18*(10), 1386–1396. https://doi.org/10.1016/S1470-2045(17)30621-6

Vaux, D. L. (2014). Historical perspective: The seven ages of cell death research. In H. Wu (Ed.), *Cell death: Mechanism and disease* (pp. 1–14). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4614-9302-0_1

Völler, D., Reinders, J., Meister, G., & Bosserhoff, A.-K. (2013). Strong reduction of AGO2 expression in melanoma and cellular consequences. *British Journal of Cancer*, *109*, 3116.

Vucic, D. (2014). IAP proteins and their therapeutic potential. In H. Wu (Ed.), *Cell death: Mechanism and disease* (pp. 97–119). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4614-9302-0_5

Walz, A. L., Ooms, A., Gadd, S., Gerhard, D. S., Smith, M. A., Guidry Auvil, J. M., Meerzaman, D., Chen, Q.-R., Hsu, C. H., Yan, C., Nguyen, C., Hu, Y., Bowlby, R., Brooks, D., Ma, Y., Mungall, A. J., Moore, R. A., Schein, J., Marra, M. A., Huff, V., Dome, J. S., Chi, Y.-Y., Mullighan, C. G., Ma, J., Wheeler, D. A., Hampton, O. A., Jafari, N., Ross, N., Gastier-Foster, J. M., & Perlman, E. J. (2015). Recurrent DGCR8, DROSHA, and SIX homeodomain mutations in favorable histology Wilms tumors. *Cancer Cell*, *27*(2), 286–297. https://doi.org/10.1016/j.ccell.2015.01.003

Wang, B., Hsu, S. H., Wang, X., Kutay, H., Bid, H. K., Yu, J., Ganju, R. K., Jacob, S. T., Yuneva, M., Ghoshal, K. (2014). Reciprocal regulation of microRNA-122 and c-Myc in hepatocellular cancer: Role of E2F1 and transcription factor dimerization partner 2. *Hepatology*, *59*(2), 555–566. https://doi.org/10.1002/hep.26712

Wang, W., Ji, G., Xiao, X., Chen, X., Qin, W.-W., Yang, F., Li, Y.-F., Fan, L.-N., Xi, W.-J., Huo, Y., Wen, W.-H., Yang, A.-G., & Wang, T. (2016). Epigenetically regulated miR-145 suppresses colon cancer invasion and metastasis by targeting LASP1. *Oncotarget*, *7*(42), 68674–68687. https://doi.org/10.18632/oncotarget.11919

Xiao, J., Lin, H., Luo, X., Luo, X., & Wang, Z. (2011). miR-605 joins p53 network to form a p53: miR-605:Mdm2 positive feedback loop in response to stress. *The EMBO Journal*, *30*(3), 524. https://doi.org/10.1038/emboj.2010.347

Yamakuchi, M., Lotterman, C. D., Bao, C., Hruban, R. H., Karim, B., Mendell, J. T., Hsuo, D., & Lowenstein, C. J. (2010). p53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. *Proceedings of the National Academy of Sciences*, *107*(14), 6334. https://doi.org/10.1073/pnas.0911082107

Yi, R., Qin, Y., Macara, I. G., & Cullen, B. R. (2003). Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes & Development*, *17*(24), 3011–3016. https://doi.org/10.1101/gad.1158803

Zhang, H., Li, Y., Huang, Q., Ren, X., Hu, H., Sheng, H., & Lai, M. (2011). miR-148a promotes apoptosis by targeting Bcl-2 in colorectal cancer. *Cell Death & Differentiation*, *18*, 1702. https://doi.org/10.1038/cdd.2011.28

Zhang, L., Huang, J., Yang, N., Greshock, J., Megraw, M. S., Giannakakis, A., Liang, S., Naylor, T. L., Barchetti, A., Ward, M. R., Yao, G., Medina, A., O'Brien-Jenkins, A., Katsaros, D., Hatzigeorgiou, A., Gimotty, P. A., Weber, B. L., & Coukos, G. (2006). MicroRNAs exhibit high frequency genomic alterations in human cancer. *Proceedings of the National Academy of Sciences*, *103*(24), 9136. https://doi.org/10.1073/pnas.0508889103

Zhang, Y., Liao, J., Zeng, S. X., & Lu, H. (2011). p53 downregulates Down syndrome-associated DYRK1A through miR-1246. *EMBO Reports*, *12*(8), 811. https://doi.org/10.1038/embor.2011.98

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